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**Intraventricular or Intracerebral Administration of PD-135,158 in CD-1
Mice:
Immediate and Long Term Behavioural Evaluation in a Modified Transition
Paradigm**

By

N. Jill Marsh, B.Sc.

**A thesis submitted to
The Faculty of Graduate Studies and Research
in partial fulfilment of
the requirements for the degree of**

**Master of Science
Specialization in Neuroscience**

Department of Psychology

**Carleton University
Ottawa, Ontario
January 2001**

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**Dr. K. Matheson, Chair
Department of Psychology**

Dr. R.M. Zacharko, Supervisor

**Carleton University
January 2001**

Abstract

The acute effects of intraventricular administration of the cholecystokinin-B receptor antagonist PD-135,158 (0.0, 0.001, 0.01 or 0.1 µg delivered in a 1 µl volume) were assessed among CD-1 mice in the modified corridor transition test apparatus. Mice received a sham injection and were re-exposed to the apparatus 3, 6, 9, and 12 weeks following drug administration (Experiment 1). Intraventricular PD-135,158 administration differentially affected behavioural indices in a dose- and time-dependant manner. In particular, the 0.001 µg dose of PD-135,158 increased anxiety-like behaviour as measured by activity, open corridor time, corridor rearing and dark chamber stretch attend postures 6 weeks post pharmacological challenge. In Experiment 2, PD-135,158 (0.0 or 0.001 µg delivered in a 1 µl volume) was administered into either the nucleus accumbens or the central nucleus of the amygdala of CD-1 mice with identical behavioural assessment. Regional differences in the behavioural as well as the temporal response to drug challenge were observed. In particular, intra-accumbal injection of PD-135,158 produced Immediate anxiogenic effects on open corridor stretch attend postures that did not emerge from intra-amygdaloid injection until 9 weeks following pharmacological challenge. Some intraregional differences in CD-1 mice receiving saline were also observed. The implications of these data for the assessment of the influence of a centrally administered cholecystokinin-B antagonist and the development of an animal model of anxiety with adequate re-test ability are discussed.

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Introduction

Anxiety has been defined as an emotional state provoked by novel or potentially threatening environmental events (Hendrie et al., 1996). In some instances, attempts have been made to differentiate anticipatory anxiety from behavioural responsivity to stressors (Lister, 1990; File, 1993). Neurochemically, the induction or enhancement of anxiety has been associated with perturbations of diverse neurotransmitter and putative neurotransmitter agents (i.e., colocalized neuroactive peptides). To a considerable degree, the relative contribution of neurotransmitter and/or neuroactive peptide alterations to the behavioural expression of anxiety has been elucidated through pharmacological interventions as well as the induction of neurochemical alterations following stressor exposure (e.g., footshock, restraint, immobilization). In the latter instance, anxiety induction in response to encounter with a stressor appears to vary according to the nature (Inoue et al., 1993) and severity (Deutch & Roth, 1990; Shibasaki et al., 1995) of the imposed stressor, prior stressor history (Davis et al., 1993; Yoshioka et al., 1995) and genetic variables (Shanks et al., 1991; Rodgers & Cole, 1993) among others. However, it is not certain whether stressor controllability per se contributes to differential release of neurochemical agents associated with behavioural expression of anxiety. It is intriguing, nevertheless, that at least one laboratory demonstrated that perceived control over stressful life events has been associated with release of endogenous anxiolytic agents (Drugan et al., 1994), which was not revealed among animals exposed to an inescapable stressor. Interestingly, central release of anxiogenic substances induced by pharmacological challenge or stressor imposition has been demonstrated in specific brain sites and selective sub-nuclei of these identical areas

(Deutch et al., 1985; Inoue et al., 1993). Likewise, paradigms which have taken advantage of the innate aversion of animals to illuminated arenas (Crawley, 1981; Costall et al., 1989; Cancela et al., 1995), open spaces (Pellow & File, 1986; Handley & Mithiani, 1994; Dawson & Tricklebank, 1995), encounters with conspecifics (Zangrossi & File, 1992a) or intruders (File & Hyde, 1979; File et al., 1985) have in some instances revealed central regional and intra-regional variability in neurochemical responsivity. Taken together, these data suggest that pharmacological challenge, stressors and specific paradigms activate distinct neurochemical systems in diverse brain sites and provide evidence for a variable spectrum of behavioural responsivity. It should be underscored that the sensitivity of central sites to exogenous stimulation is likely associated with the neurochemical mosaic of specific brain regions. In addition, the interactive influence of multiple anxiogenic agents released in diverse central sites to the elicitation of behavioural change is not precluded. In effect, expression of specific behavioural symptoms of anxiety may well follow from the differential reactivity of central sites to particular environmental events and the endogenous release of anxiogenic substances. In this respect, a variety of central anxiogenic agents have been identified including cholecystokinin (CCK) (Harro & Vassar, 1991; Hughes et al., 1991; Rex et al., 1994; Beinfeld, 1995), corticotropin releasing factor (CRF) (Berridge & Dunn, 1989; Liang & Lee, 1988) yohimbine (Handley & Mithiani, 1984; Charney & DeRedmond, 1983) and beta-carboline (Prado de Carvalho et al., 1983; File & Lister, 1984) among others. The identification of clinically relevant panic symptoms following systemic CCK administration in human subjects (Bradwejn et al., 1990; Bradwejn & Koszycki, 1994) and the anxiogenic influence of CCK in rats (Harro & Vassar, 1991; Rex et al., 1994) and

mice (Hagino, Moroji & Iizuka, 1989) have prompted considerable research. Moreover, the localization of CCK in various central sites associated with attention, arousal and affect (Fekete et al., 1984; Singh et al., 1991; Derrien et al., 1993; Belcheva et al., 1994) has suggested involvement of this peptide in the responsivity of organisms to diverse stressful life events.

Cholecystokinin

Cholecystokinin was originally identified as a gut hormone participating in satiety signals (Ivy & Oldberg, 1928) but has also been detected within the mammalian central nervous system (CNS) (Vanderhaeghen et al., 1975; Beinfeld et al., 1981). For example, CCK has been implicated in pain perception (Baber et al., 1989), sedation (Zetler, 1985) and satiety (Gibbs, Young & Smith, 1983) as well as learning and memory (Gall et al., 1987; Itoh et al., 1989; Harro & Vasar, 1991). Cholecystokinin was initially described as a 33 amino acid peptide (Jorpes & Mutt, 1966; Mutt & Jorpes, 1968), but several molecular variants of CCK have been identified, including a 39- (Eysselein et al., 1984), a 47- (Johnson & Rehfeld, 1992), and a 58- (Eberlein et al., 1988) amino acid form. Synthesis of these fragments from the prepro-CCK molecule involves a series of post-translational processing stages and peptide cleavage (Turkelson et al., 1990; Blanke et al., 1993). Nevertheless, currently identified molecular fragments of CCK with demonstrated central and peripheral activity display an identical eight amino acid sequence at the carboxyl portion of the peptide. This C-terminal sulphated octapeptide is rigorously conserved across species (Crawley et al., 1984). In rats and mice, CCK fragments with demonstrable influence on behaviour include CCK-8-sulfated (CCK-8S) (Dauge et al.,

1989; Derrien et al., 1993), CCK-8-unsulfated (CCK-8US) (Fekete et al., 1984) and CCK-4 (Harro & Vassar, 1991; Rex et al., 1994), although the sulphated octapeptide (CCK-8S) predominates in brain tissue (Albus, 1988). CCK-8US and CCK-4 are preferential CCK-B receptor agonists, while CCK-8S binds non-selectively to CCK-A and CCK-B receptors (Hamilton & Freeman, 1995). Degradation of these forms of CCK appears to occur through a membrane-bound neutral endopeptidase also associated with the degradation of several other peptides including enkephalins and angiotension (Deschodt-Lanckman, 1982; Zuzel et al., 1985; Najdovski et al., 1987).

Cholecystokinin fragments have been noted to exert differential behavioural effects as a function of dose and route of administration. For example, CCK-8S may experience considerable difficulty crossing the blood brain barrier. Accordingly, CCK-8S is typically administered intracerebrally or intraventricularly among infrahuman subjects (Zhu et al., 1986). In contrast, the CCK-4 tetrapeptide readily crosses the blood brain barrier (Kastin et al., 1970). Indeed, the potency of systemic CCK-4 administration in eliciting behavioural effects in clinical settings (e.g., Bradwejn & Koszycki, 1994) and the ease of this route of administration among infrahuman subjects (e.g., BOC-CCK-4, Gerhardt et al., 1994) have provided a potentially heuristic animal model of anxiety. It should be underscored that CCK-4 exerts only weak peripheral CCK-A receptor activation and presumably activates central CCK-B receptors selectively (Hamilton & Freeman, 1995). As will be elucidated in ensuing sections, diverse CCK peptide fragments possess differential abilities to exert central and peripheral effects, to activate specific CCK receptor subtypes and to influence specific subsets of behaviour as a function of the route of drug administration (see Banks & Kastin, 1996 for review).

In rats and mice, conspicuous concentrations of CCK have been detected in the cerebral cortex, amygdala, hypothalamus and the raphe nuclei (Beinfeld et al., 1981; Lotstra & Vanderhaeghen, 1987; Hokfelt et al., 1988). Interestingly, CCK is colocalized with a number of neurotransmitters, most notably dopamine (DA) in the mesolimbic pathway in rats and mice (Hokfelt et al., 1980; Studler et al., 1981; Seroogy et al., 1989). Cholecystokinin also coexists with several neuroactive peptides including substance P in the central grey (Skirboll et al., 1982), enkephalin in the hippocampus (Vanderhaeghen et al., 1980; Gall et al., 1987), and oxytocin and/or corticotropin-releasing hormone within the paraventricular nucleus of the hypothalamus (Vanderhaeghen et al., 1980). In the VTA, DA neurons containing CCK innervate the posterior medial nucleus accumbens, the medial olfactory tubercle and the medial septum (Hokfelt et al., 1980; Studler et al., 1981; Seroogy et al., 1989). Conversely, DA projections from the VTA to the anterior nucleus accumbens and caudate are non-CCK-containing DA pathways (Hokfelt et al., 1980). Approximately 30% of the CCK detected in the anterior accumbens and caudate nucleus appears to be derived from the amygdala, while the remaining innervation is derived from the claustrum and the piriform cortex (Meyer et al., 1982). It would appear that in some instances central application of CCK influences neuronal firing rates and release of colocalized neurotransmitters reminiscent of those provoked by environmental stimuli. For example, microiontophoretically applied CCK in the VTA increases the depolarisation of DA neurons (Hommer et al., 1986; Freeman & Chiodo, 1988), and intracerebral injection of nanogram concentrations of CCK into the A10 area potentiates DA-induced hypolocomotion (Crawley, 1989). In addition, in the nucleus accumbens, CCK potentiation of the inhibitory actions of DA are mediated by the CCK_A receptor

(Kelland et al., 1991), while the excitatory neuronal influence of CCK follows from CCK_B receptor activation (Hommer et al., 1986). The opposing influence of CCK_A and CCK_B receptor activation on central DA activity has also been demonstrated behaviourally (Josselyn & Vaccarino, 1996). Indeed, systemic administration of the CCK_B receptor antagonist, L-365,260, increased the acquisition of DA-mediated conditioned locomotor activity, whereas administration of devazepide, a CCK_A receptor antagonist, impaired acquisition of conditioned locomotor activity. In effect, the contribution of CCK in influencing DA associated behavioural alteration appears to be dose dependent (Cohen et al., 1982; Ferrer et al., 1988; Britton et al., 1989), route specific (e.g., intraventricular cf. intraperitoneal) (DeWitte et al., 1988; Khosla & Crawley, 1988; Hamilton et al., 1990) and displays brain region variability (Ladurelle et al., 1993). Taken together, CCK appears to exert stressor-like effects and to provoke behavioural, neuronal and neurochemical alterations reminiscent of aversive life events. Although this discussion focuses on a DA/CCK interaction in select mesolimbic sites, there is no a priori reason to suspect that such a neurochemical interaction provides an exclusive operational definition of anxiety, precludes neurochemical interaction with other central sites or defines the nature of the anxiety expressed. For example, there is sufficient evidence to suggest that CCK interacts with norepinephrine (NE) in the locus coeruleus (Kalivas, 1985) and serotonin (5-HT) in the raphe system (Sim & Joseph, 1993) to provoke behavioural indices of anxiety. Taken together, these data suggest that the central influence of CCK in response to environmental challenge is unique to the central site in which the peptide is released as well as the neurotransmitter with which it is colocalized.

To date, identification of the CCK_A and the CCK_B receptors previously documented in the nucleus accumbens has provided a functional framework for the description of anxiety-associated behaviours, although the nature of this interface remains enigmatic. The CCK_A receptor was initially classified as a peripheral receptor in the alimentary tract with considerable affinity for the CCK-8S ligand, although CCK-8S has comparable affinity for both the CCK_A and the CCK_B receptor; while the CCK_B receptor was detected centrally and found to be relatively selective for CCK-4 and CCK-8US (Hill et al., 1987; Lotti & Chang, 1989; Hill & Woodruff, 1990). Recently, radioligand binding and molecular genetic analyses have provided evidence for conspicuous concentrations of central as well as peripheral CCK_A receptors (Moran et al., 1986). While selective CCK_A receptor agonists are currently unavailable, a number of relatively specific CCK_A receptor antagonists have been developed (e.g., lorgumide and proglumide, the benzodiazepine derivative devazepide). Central CCK_B receptors activated by CCK-4 and the peptidase-resistant CCK derivative, BOC-264, are widely distributed in the rat and mouse, particularly in nigrostriatal, mesolimbic and mesocortical sites (see Crawley & Corwin; 1994 for review). For example, significant CCK_B receptor densities have been described throughout the nucleus accumbens (i.e., core and shell in a rostral-caudal orientation), the amygdaloid nuclei and the ventral tegmental area. Central CCK_A receptor distribution appears to be more discretely organized relative to the CCK_B receptor configuration. In particular, CCK_A receptors have been localized primarily in the anterior nucleus accumbens, hypothalamus and nucleus tractus solitarius (Moran et al., 1986) in rats and mice. In addition, species-specific pharmacological characteristics of CCK_B receptors have been described in a

number of investigations (e.g., Kuwahara et al., 1993; Derrien et al., 1994). In particular, Kuwahara et al. (1993) reported that the mouse CCK_B receptor had a higher affinity for CCK-4 than rat, guinea pig and human CCK_B receptors. This finding is not a trivial one although the implications of this demonstration have not gained prominence in the literature. For example, at the very least, these data suggest a hierarchy of anxiogenic influence predicated on the environmental challenge imposed. Delineation of such an anxiogenic hierarchy across species for the various CCK fragments could potentially provide a heuristic clinical analogue. It should also be noted that inter-species (Hinks et al., 1995) differences in CCK receptor distribution and response to stressor imposition have been described. For example, Hinks et al. (1995) demonstrated clear differences in hypothalamic CCK receptor responsivity to the metabolic stressor of a physiological saline challenge in the rat and mouse. In particular, a six-day schedule of systemic 2% sodium chloride administration resulted in a conspicuous increase in CCK_B receptor binding but provoked a negligible increase in hypothalamic CCK_A receptor binding in the rat. This identical stressor in mice, in contrast, provoked a selective enhancement of hypothalamic CCK_A receptor binding. In addition, autoradiography and in situ hybridization revealed changes in central CCK_A and CCK_B receptor mRNA, demonstrating that CCK_A and CCK_B receptor density within the rodent hypothalamus is altered in response to a chronic physiological stressor. In effect, dynamic variations in CCK receptor availability in diverse central sites following encounter with putative anxiogenic stimuli may alter subsequent responsivity to ensuing stressors. The putative influence of endogenous variations of central CCK_B receptor densities upon behaviour has also been evaluated. In 1990, Harro et al. detected higher CCK_B receptor densities in

the medial prefrontal cortices of rats exhibiting anxious behaviours in the elevated plus maze as compared to non-anxious counterparts. The preceding arguments clearly reveal that central responsivity to CCK_A or CCK_B receptor activation may be stimuli and site specific. The rather restricted central distribution of the former receptor relative to the CCK_B receptor as well as an opposing biological influence suggests that incorporation of immediate sensory information may occur in selective central sites while other sites may contribute to the sensitization of stressor specific behaviours. For example, the nucleus accumbens has been identified as a central site with both CCK_A and CCK_B receptors and is associated with integration of sensory information and subsequent motivational behaviour (Phillips et al., 1991) while the nucleus tractus solitarius, linked to memory formation of stressful events (Walker & Davis, 1997) exhibits only CCK_A receptor density (Hokfelt et al., 1991). Some clinical investigators have in fact suggested that the relative activation of central CCK A and B receptors underlie expression of anxiety as well as responsivity to therapeutic interventions (Bradwejn & Koszycki, 1994). Indeed, a putative relationship between the nucleus accumbens, the amygdala and the nucleus tractus solitarius may be delineated. For example, the nucleus accumbens may participate in organizing the motivational status of the animal relative to an attending stressor, the amygdala may provide an affective colouring to the immediate stressor experience while the nucleus tractus solitarius conceivably integrates the visceral memory of a stressor and influences prospective encounters with similar events (see Cahill & McGaugh, 1998). The interaction of such sites, the dynamic nature of CCK receptor density, and the particular CCK fragment released, coupled with the influence of some stressor variables

(e.g., severity, controllability, predictability) may provide a unique response to present or future stressor imposition.

It will be recalled that CCK has been identified as a potent anxiogenic substance following systemic administration in human subjects (Bradwejn et al., 1990; Bradwejn et al., 1992; Bradwejn & Koszycki, 1994) as well as central and peripheral challenge among infrahuman subjects (Derrien et al., 1993; Acosta, 1998). However, CCK receptor subtypes may influence responsivity to anxiety provoking environmental challenge and elicit specific behavioural symptoms as a function of the central sites activated by the stimulus. The CCK-4 fragment conspicuously linked to anxiety-like behaviour in animals and panic symptoms in humans has demonstrable affinity for the central CCK_B receptor. In human volunteers, intravenous administration of CCK-4 rapidly induces panic-like symptoms (Bradwejn et al., 1992). In animals, relatively abbreviated exposure of rats to the odour of a predator (e.g., a cat) increases concentrations of CCK-4 in the mesocortex and the central amygdaloid nucleus (Pavlovic et al., 1993). In contrast, elevated concentrations of CCK-8 in response to predator-associated cues were only evident in the nucleus accumbens. Rex et al. (1994) investigated the behavioural effects of acute systemic administration of CCK-4 in the elevated plus maze, the light/dark box, a conflict test in adult male rats and the ultrasonic vocalisation paradigm in five-day-old rat pups. Interestingly, CCK-4 increased anxiety scores in the respective animal models of anxiety employed, while no evidence of augmented anxiety was found following systemic CCK-8S administration. Unfortunately, such a demonstration fails to consider the suspected limited central accessibility of peripherally injected CCK-8S. While it is obvious that CCK is intimately involved in an organism's response to an anxiogenic episode or a

stressor, particular CCK fragments may be specifically involved in organismic responsivity to a particular stressor. Indeed, it is probable, that certain CCK fragments may provoke differential response to stimuli in an inter-regionally dependent manner.

Cholecystokinin has been demonstrated to influence learning and memory in diverse behavioural paradigms by influencing a number of central sites (Gall et al., 1987; Itoh et al., 1989; Harro & Vasar, 1991). In this respect, a rather prominent role for endogenous CCK activity has been verified in the hippocampus (Nunzi et al., 1985). In particular, CCK immunoreactivity in the hippocampus appears to be associated with heightened activity of pyramidal neurons (Groner & Bebonnel, 1995) implicated in the modulation of GABA-dependent memory processes (Hendry & Jones, 1985). Further to this point, the CCK_A receptor antagonist, devazepide, has been shown to produce amnesic effects in an olfactory recognition test in rats, apparently diminishing the saliency of the task, while the CCK_B receptor antagonist L365260 has a facilitatory effect on social recognition (Lemaire et al., 1992). In effect, manipulations of the central CCK_A and CCK_B receptors may influence the formation of some types of memory.

In evaluating the anxiogenic influence of CCK in animal models of anxiety, the influence of CCK in promoting variations in ingestive behaviours should be addressed owing to the potentially confounding influence of perturbations in consummatory behaviour. Indeed, some laboratories have suggested that behavioural changes in some animal models of anxiety may be occasioned by the induction of nausea or some like gastrointestinal disturbance rather than alterations in the emotional and/or motivational status of the organism. For example, administered peripherally, low doses of CCK-33 inhibit feeding and elicit satiety behaviours in non-human primates (Mueller & Hsaio,

1977; Corwin & Schuster, 1993), rats (Gibbs, Young & Smith, 1973a; Gibbs, Young & Smith 1973b) and human subjects ordinarily associated with post-meal ingestion (Dourish, 1992), while high dose bolus injections promote physical discomfort in human subjects (Geary et al., 1992; Melton, Kissileff & Pi-Sunyer, 1992). Such effects appear to be mediated by CCK-A receptors since satiety-associated behaviours promoted by CCK-33 administration are attenuated by peripheral CCK_A but not CCK_B antagonist administration (Dourish et al., 1989; Moran et al., 1992). In contrast to the influence of peripheral drug administration on ingestive behaviour, the effects of central administration of CCK receptor antagonists on feeding are ambiguous. For example, the reinforcing influence of satiety provoked by reductions of gastric motility and glucoregulatory variations need to be contrasted with parallel variations in emotional state (cf. Puglisi-Allegra et al., 1991). It is imperative, at this juncture, to emphasise that conclusions pertaining to CCK and behavioural output is intimately associated with the site of drug administration, the nature of the stimulus and the density and distribution of CCK_A and CCK_B receptors. It is not surprising that functions such as learning and memory, which include components of novelty and the physiological stressor of food deprivation (e.g., glucoprivation), induce comparable, overlapping or shared aspects of anxiety.

Clearly, prominent behavioural correlates of central CCK activation are in evidence following CCK_B receptor manipulations (Hughes et al., 1990; Costall et al., 1991). In contrast, CCK_A receptor antagonists provide ambiguous or perhaps subtle behavioural alterations in infrahuman paradigms. To be sure, lack of selective CCK_A receptor agonists complicates attempts to delineate the specific contribution(s) of CCK_A

receptors to changes in anxiety-like behaviours. In animal models of anxiety, there is a lack of data to describe the site-specific neurochemical effects of anxiogenic agents including CCK in mesocortical and mesolimbic sites. As well, the paucity of brain site-specific research pertaining to the anxiogenic influence of specific CCK fragments precludes detailed documentation of the functional impact of CCK in specific central regions. In particular, CCK availability in diverse brain areas associated with anxiety-like behaviours (e.g., CCK within the amygdala may contribute to affective aspects of an anxiety-provoking situation, Benkelfat et al., 1995) suggests that CCK may affect variable behavioural responsivity following stressor imposition. In addition, demonstrated differences in receptor density as well as the often-opposing effects provided by the differential activation of CCK_A and CCK_B receptors, preclude a simple prediction of the global response of an organism to a stressful event. Such neurochemical overlap may provide organisms with multiple behavioural responses and diverse reactivity to anxiogenic environmental events.

Animal Models of Anxiety

The role of CCK in the induction of anxiety-like behaviour has been intensively examined in diverse animal models. Behavioural responsivity to specific environmental challenge appears to follow from the nature of the stimulus encountered and the interplay among transmitters and putative transmitters in specific central sites (see Zacharko et al., 1995 for review). Diverse models of anxiety have been employed among infrahuman subjects in paralleling human psychopathology as well offering putative prophylactic and therapeutic intervention strategies. Such infrahuman paradigms can be characterized as

those utilising conditioned responses, including conflict (Geller et al., 1963), startle (Davis, 1979), defensive burying (Treit et al., 1981), or exploration (e.g., hole board task, File & Wardill, 1975) or those tasks which capitalize on the innate aversion of animals to illuminated arenas in the light/dark paradigm (Crawley, 1991) or the open arms of the elevated plus maze (Montgomery, 1958). The latter paradigms in rats and mice induce anxiety following encounter with novelty or a potentially aversive or threatening stimulus (e.g., bright light) that may parallel clinical forms of anxiety engendered by stressful or uncontrollable life events. The topography of the elevated plus maze, for example, can induce variations in the profile of anxiogenic responses in animals due to the presentation of a potentially threatening novel environment (Rodgers & Cole, 1994; Dawson & Tricklebank, 1996).

The elevated plus maze, typically employed in rats and mice, consists of opposing open and closed arms within a raised apparatus. The lack of activity of animals in the open arms of the elevated plus maze and the confinement of behaviour to the enclosed arms of the maze has been associated with hormonal (Adamec et al., 1991) and behavioural changes (Rodgers et al., 1992; Zangrossi & File, 1992b; Shepherd et al., 1994) consistent with an operational definition of anxiety, characterized by elevated plasma corticosterone concentrations (Pellow et al., 1985), instances of freezing, production of faecal boli, and stretch attend postures (Pellow et al., 1985; Moser, 1989; Lee & Rodgers, 1990). Alterations of exploratory behaviour in the closed arms of the elevated plus maze are consistent with the influence of systemic and central administration of various anxiogenic substances that provoke dose dependent behavioural profiles of anxiety. Conversely, anxiolytic agents reduce the innate aversion of animals

to the open arms of the apparatus (see Rodgers and Cole, 1994 for review). Accordingly, conventional assessment of anxiety-like behaviour in the elevated plus maze in rats and mice has focused on open arm entries and cumulative time spent in the open portions of the apparatus. The total or closed number of arm entries (Pellow et al., 1985) has been argued to provide a measure of locomotor activity. The propensity of the elevated plus maze to provide an index of activity in conjunction with positional indices of anxiety favours an analysis that is not compromised by apparatus variables or pharmacological interventions. In spite of the widespread utilisation of the plus maze as an animal model of anxiety, the sensitivity of this task has been burdened by a plethora of apparatus cues, which provoke variable neurochemical and behavioural effects. For example, subtle variations of maze illumination (Griebel et al., 1993) and the presence or absence of raised edges in the open arms of the maze (Fernandes & File, 1996) provoke appreciable alterations of inter-laboratory measures of anxiety. In addition, organismic and experiential variables impact considerably on performance in the elevated plus maze. To be sure, strain variables, social isolation, pre-test handling and operational definitions of open arm entries provide additional sources of variability in this paradigm (see Hogg, 1996 for review). Clearly, some of these caveats have detracted from the perceived efficacy of the elevated plus maze in assessing anxiety. In addition, the a priori assumption that the validity of the elevated plus maze is compromised owing to therapeutic failure of benzodiazepine administration on re-test is a fallacious one (but see Lister, 1987; File, 1990; Rodgers et al., 1992). Such an argument is inconsistent with the identification of graded levels of anxiety (File, 1996), the differential pharmacological sensitivity of anxiety-like behaviours (Dawson & Tricklebank, 1995) and the apparent

sensitivity of diverse central sites to anxiogenic and anxiolytic drug administration (Handley et al., 1993).

The performance of animals in the light/dark paradigm is, in some respects, reminiscent of the behaviour of rats and mice in the elevated plus maze. The light/dark apparatus consists of an illuminated chamber with access to an unlit and presumably safe area (Crawley & Davis, 1982). Ordinarily, rats and mice demonstrate a conspicuous preference for the protected aspect of the apparatus (Crawley, 1981; Kilfoil et al., 1989). Pharmacological strategies that attenuate preference for the protected portion of the light-dark box are considered to provide anxiolytic influence, and the light/dark box has been employed in the identification of putative anxiolytic agents. Anxiolytic agents increase transitions between the adjoining portions of the arena, exploratory activity, time spent in the lit area, and decrease freezing and stretch attend postures (Crawley & Goodwin, 1980; Costall et al., 1988; Onaivi & Martin, 1989; Young & Johnson, 1991a,b; Lopez-Rubalcava et al., 1992). Systemic and central administrations of anxiogenic agents promote behaviours opposed to those of anxiolytic drug administration (Costall et al., 1988; Crawley et al., 1984; Young and Johnston, 1991a,b). Behavioural alterations afforded by drug administration and particularly preference for the unlit area presumably follow from altered perception of risk assessment in a novel environment (see Crawley, 1985 for review). However, in the light/dark paradigm, some anxiolytic drugs selectively increase exploratory activity or time spent in the lit areas of the apparatus without affecting transitions between the light and the dark compartments (Onaivi & Martin, 1989; Young & Johnson, 1991a,b). In addition, measures of locomotor activity are compromised in the light/dark task (Onaivi & Martin, 1989; Young & Johnson, 1991a,b).

Taken together, the light-dark task employs a restricted set of behavioural indices and the nature of the paradigm prevents detection and/or elaboration of subtle behavioural differences occasioned by pharmacological challenge. These paradigm specific limitations are exacerbated when dose, route and the site of drug administration are varied and evaluated against a variable experiential backdrop (e.g., re-test experience, pre-test handling).

Current animal models of anxiety attempt to assess the propensity of the paradigm to monitor dynamic variations in anxiety following benzodiazepine administration. To be sure, the efficacy of benzodiazepine treatment in attenuating or modifying expression of anxiety-like behaviour has been reliably demonstrated in the elevated plus maze (see Rodgers and Cole, 1994 for review) and the light-dark paradigm (Crawley & Goodwin, 1980; Crawley, 1981). However, response repertoires in anxiety provoking paradigms are influenced by past experience (Davis et al., 1993; Yoshioka et al., 1995). Accordingly, memory of anxiogenic episodes or a series of such experiences should influence anticipation of and subsequent response to encounters reminiscent of such prior encounters. While benzodiazepine administration fails to attenuate expression of anxiety in paradigms involving repetitive testing (File, 1990), such pharmacological inadequacies address the issue of neurochemical correlates of behavioural change rather than perceived limitations of the paradigm under investigation. To be sure, there are sufficient data which indicate that the nature of a stressor, the severity of the aversive encounter as well as the predictability of the stressful life event influence the profile of neurochemical alteration in diverse central sites (see Weiss, 1991; Roth et al., 1988; Chaouloff, 1993 respectively). In effect, putative non-benzodiazepine anxiolytics, including CCK_B

receptor antagonists, as well as pharmacological strategies that influence CCK-DA associated correlates of anxiety may provide indices of behavioural alterations that are resistant to benzodiazepine treatment owing to anxiety associated neurochemical change. Taken together, it should be considered that exposure of animals to anxiogenic stimuli induce stressor-like neurochemical alterations that are subject to conditioning and/or sensitization (Antelman, 1988; Robinson, 1988; Antelman & Caggiula, 1990; Paulson, Camp & Robinson, 1991)

Issues pertaining to central and peripheral routes of putative anxiolytic and anxiogenic drug administration and the dose specific influence of such interventions complicate consideration of neurochemically distinct and benzodiazepine-resistant forms of anxiety. Cholecystokinin provokes prominent respiratory (Pagani et al., 1982; Gillis et al., 1983), cardiovascular (Pagani et al., 1982) and thermotactic (Katsuura & Itoh, 1981; Morley, Levine & Lindblads, 1981) responses following systemic administration. Some of these peptide associated neurovegetative alterations appear to follow from peripherally derived neural alterations that may obscure central influence (Pagani et al., 1982; Hurle et al., 1985). Curiously, relatively few laboratories have employed central administration of CCK agonists and antagonists in the assessment of behavioural profiles attending animal models of anxiety. Investigations which have favoured central CCK agonist and antagonist challenge in behavioural paradigms have typically employed high doses of the peptide which precludes a systematic assessment of response thresholds or the detection of graded behavioural change. Of greater concern, perhaps, is the adoption of intracerebroventricular procedures that seriously compromise the reliability and/or the efficacy of the pharmacological agent employed. For example, it is not uncommon to find

microliter injection strategies favouring ventricular distension and mechanical trauma owing to the rate of drug administration or to the volume injected (Hong et al., 1995). In conclusion, paradigms employing central administration of minimally effective doses of anxiogenic agents are lacking. Moreover, there are no current data provided in an animal model of anxiety that describe behavioural responsivity to repeated exposure to a relatively mild psychological stressor.

Clearly, although the elevated plus maze and the light-dark paradigm provide some advantages to behavioural and pharmacological intervention strategies, there is an obvious need to develop alternate paradigms that assess a broader spectrum of behavioural change. For example, Shimada et al. (1995) introduced a paradigm that offers a mildly stressful experience and higher behavioural degrees of freedom relative to the challenge afforded by the elevated plus maze or the light/dark box. The adoption of this corridor transition test apparatus provides measures of locomotor activity and exploration (see Figure 1). Behavioural indices of anxiety provided by Shimada et al. (1995) included exploratory transition frequency between the illuminated corridors and the dark chambers, time spent by the mice in the lit corridors as well as locomotor activity. The systemically administered benzodiazepine anxiolytics, diazepam and alprazolam, dose dependently increased the aforementioned behavioural scores induced by initial paradigm exposure, while the anxiogenic substances, picrotoxin and beta-carboline decreased behavioural scores in these identical anxiety indices. While the corridor apparatus introduced by Shimada et al. (1995) appears sensitive to benzodiazepine associated intervention strategies, the potential anxiolytic influence of CCK_B derived receptor antagonists in attenuating such mild stressor experiences has not

been examined. Likewise, the long-term consequences of initial mild anxiogenic experience and subsequent reactivity to mild stressor re-exposure remain to be evaluated. Finally, it is conceivable that diverse aspects of anxiety engendered by exposure of animals to novelty may be dose dependently altered on first experience by a CCK_B antagonist. Indeed, the central site of drug administration may well influence such experiences. Intuitively, this anxiety paradigm in a repeated measures design favours analysis of the propensity of CCK alterations to effect protracted behavioural changes in anxiety.

In summary, animal models of anxiety to date have largely failed to (a) assess the short or long term influence of centrally administered CCK associated peptides (b) provide a pharmacological analysis of minimally effective doses of CCK challenge (c) provide a behaviourally specific CCK response profile attending low dose CCK challenge and (d) assess the relative impact of differential CCK receptor activation to the expression of behavioural alteration in response to a mild psychological stressor or intermittent stressor. The following series of Experiments attempted to evaluate (a) the immediate and long term behavioural consequences of central administration of CCK receptor antagonists in a modified corridor paradigm on CD-1 mice, (b) identify the minimal effective doses of a centrally administered CCK_B receptor antagonist on specific behavioural indices of anxiety within the modified corridor paradigm, and (c) the differential behavioural profiles generated through intraventricular and intraregional injection of a CCK_B receptor antagonist.

Figure 1. Modified Corridor Transition Test Apparatus

Experiment 1

Behavioural indices of anxiety in response to a mild psychological stressor following central administration of CCK_B receptor antagonists are lacking and the long-term consequences of such drug administration are likewise unavailable. It will be recalled that systemic administration of the CCK_B receptor antagonist, PD-135,158, provokes dose dependent reductions in anxiety-like behaviours in the elevated plus maze and light/dark box in rats (Costall et al., 1991), guinea pigs (Rex, Fink & Marsden, 1994) and mice (Hughes et al., 1990; Costall et al., 1991). In particular, considerable reductions in anxiety scores among mice appear with systemic doses of PD-135,158 between 0.01 and 0.1 mg/kg (Hughes et al., 1990; Costall et al., 1991). Experiment 1 was designed to (a) evaluate the putative anxiolytic effects of intraventricular administration of the CCKB receptor antagonist PD-135,158 in response to a mild psychological stressor, (b) ascertain the minimally effective dose of PD-135,158 at affecting behavioural change and (c) assess the long term effects of initial pharmacological challenge on operationalized measures of anxiety like behaviour in a modified light-dark paradigm.

Methods

Subjects

Thirty-two naive, male, CD-1 mice were obtained from Charles River (St. Constant, Quebec) at five weeks of age. Animals were acclimated to the animal facility until an acceptable age and/or weight for surgical manipulation (3 months old or 30 g) was achieved. All mice were housed four per cage and maintained on a 12-hour light-dark cycle (light on 7:00 am until 7:00 PM), with food and water available *ad libitum*.

Stereotactic Surgery

An inhaleable gas mixture of oxygen and halothane (CDMV) was employed to induce surgical anaesthesia. The mouse was placed in a David Kopf stereotaxic instrument fitted with a mouse adapter, the scalp was cleaned and an anterior-posterior incision was made to expose the skull surface. Co-ordinates used for the surgical implantation of a 23-gauge cannula into the lateral ventricle were A.P. +0.8 mm from Bregma, L. +0.7 mm from the midline and V. -2.6 mm from a flat skull surface. A 30-gauge stylette was employed to increase the patency of the implanted cannula. Following surgery, animals were housed individually, placed on a warm heating pad and provided a dietary supplement (Meritene nutritional supplement in wet crushed food pellets) in a recovery room for a period of one week. Following behavioural recovery, mice were returned to the main animal holding area for approximately 7 days until the commencement of behavioural testing.

Apparatus

The present investigation used a modified version of the apparatus introduced by Shimada et al. (1995). It consisted of two open L-shaped corridors adjoined by two dark chambers (see Figure 1). The outer (40 x 12 cm) and inner walls (20 x 12 cm) were made of white and transparent Plexiglas, respectively. The floor of the apparatus (10 cm width) was comprised of white Plexiglas and partitioned into 10 cm squares. The two dark chambers (10 x 10 x 12 cm) were constructed of black Plexiglas. Each dark chamber was linked to a passageway (3.5 x 3.5 cm) on either side allowing entry into the illuminated corridors. The apparatus was enclosed in a black waffle board enclosure (80 x 80 x 80

cm) that was illuminated by a fluorescent light source (12 W) positioned 80 cm above the centre of the apparatus. A video camera installed above the apparatus recorded the behavioural test for subsequent analysis.

Procedure

Following recovery from surgery, animals were randomly assigned to either drug or saline conditions ($n = 8/\text{cell}$). Intraventricular administration of PD-135,148 (saline, $0.1 \mu\text{g}/\mu\text{l}$, $0.01 \mu\text{g}/\mu\text{l}$ or $0.001 \mu\text{g}/\mu\text{l}$) (Research Biochemicals International, Natick, MA) was accomplished employing a Hamilton $5 \mu\text{l}$ syringe connected to a 30-gauge injector. A $1 \mu\text{l}$ volume was injected over a two-minute period and the injector left in position for an additional minute to ensure adequate drug diffusion. The stylette was then replaced. Mice were only challenged intraventricularly on the first day of behavioural testing. On subsequent tests, which were initiated at three-week intervals, all mice were handled in a manner consistent with initial intraventricular challenge but the drug was not administered. The sham procedure consisted of inserting the 30-gauge injector within the guide cannula for a three-minute period. Ten minutes following completion of the intraventricular or sham administration protocol, mice were gently placed in an open corner of the apparatus and tested over a ten-minute interval in the modified light/dark box. Behavioural scoring of the test session was divided into consecutive five-minute test blocks. For each five minute interval, behavioural measures were obtained which described (a) locomotor activity, (b) episodes of grooming, (c) time spent in the open corridors of the apparatus, (d) corridor rearing, (e) stretch attend postures from dark chambers into lit corridors, (f) stretch attend postures within the lit corridors and (g)

transition frequency between the open corridors of the test chamber. The entry into a chamber or a line crossing was defined as the placement of all four paws into the chamber or across the line. In addition, latency measures and occupancy scores were secured which described (a) latency to dark chamber entry, (b) initial corridor transition, (c) initial time in a dark chamber and (d) dark chamber re-entry. All testing was carried out between 8 a.m. and 12 p.m. Following testing, animals were returned to the main animal holding area for the three-week period between behavioural tests. A random sampling and analysis of test scores by an independent observer verified the reliability of behavioural scoring.

Following completion of the Experiment 1, animals were overdosed with sodium pentobarbital and perfused intracardially with 0.9% saline followed by a 10% formalin solution. The brains were removed from the skull, blocked, frozen and sectioned at 40 μm . Coronal brain sections were stained with cresyl violet and examined under a microscope to verify cannula placement in the lateral ventricle.

Statistical Analysis

Dependent variables measured included the behavioural measures of (a) locomotor activity, (b) episodes of grooming, (c) time spent in the open corridors of the apparatus, (d) rearing, (e) stretch attend postures from dark chambers into lit corridors, (f) stretch attend postures within the lit corridors and (g) transition frequency between the open corridors of the test chamber. The entry into a chamber or a line crossing was defined as the placement of all four paws into the chamber or across the line. In addition, several latency measures secured described (a) initial corridor transition, (b) dark

chamber return, and (c) initial time in a dark chamber and (d) dark chamber re-entry. The independent variables used to evaluate these measures were the between group variable drug dose and the within group variable of test number. Test measures from the first and second 5 minute blocks as well as the total 10 minute length of the test were analysed individually. For simplicity, only the most pertinent test portion analysis is reported in detail in the Results section of Experiment 1. Full analyses summaries can be found in Appendix A. Statistical analysis was completed using SPSS 7.0 analysis of variance repeated measure procedure using an alpha level of 0.05 for tests of the main and interaction effects. A priori comparisons between the drug groups and the saline control condition was made using an alpha level of 0.05 and appropriate post hoc multiple comparisons were evaluated within significant main effects and/or interactions using Bonferroni and Ryan Einot Gabriel & Welsch F (REGW-F) tests. Violations of the sphericity assumption for within-subjects effects were treated by adjustment to critical degrees of freedom using Greenhouse-Geisser and Huynh-Feldt corrections as recorded in Appendix A.

Results

Microscopic examination of the coronal brain sections of mice included in Experiment 1 confirmed accurate placements within the lateral ventricle ($N = 32$). Moreover, intraventricular injection did not appear to result in observable tissue damage. The data pertaining to some intraventricular placements among CD-1 were discarded owing to inaccurate placements ($n = 9$), severe ventricular distortion ($n = 7$) or apparent haemorrhaging ($n = 6$) in and around the cannulation site.

Activity

An analysis of variance of the activity scores, operationalized by apparatus line crossings within the apparatus, among CD-1 mice challenged with 0.001 μ g, 0.01 μ g, 0.1 μ g PD-135,158 or saline revealed a significant main effect of Week ($F_{(4,112)}=12.167$, $p<.05$) and a significant Week x Drug interaction ($F_{(12,112)}=2.622$, $p<.05$), but failed to reveal a significant main effect of Drug ($F_{(3,28)}=0.716$, $p>.05$) for the first 5 minutes of the test period. Simple main effects analysis and REGW-F multiple comparisons ($\alpha=0.05$) identified a significant Drug effect 6 weeks post drug administration ($F_{(3,28)}=3.172$, $p<.05$). In particular, although not significantly different from saline challenged mice, CD-1 mice previously administered 0.1 μ g PD-135,158 were more active than mice previously challenged with 0.001 μ g or 0.01 μ g PD-135,158 (see Figure 2).

 Insert Figure 2 about here

Grooming

An analysis of variance of the grooming scores of CD-1 mice challenged with 0.001 μ g, 0.01 μ g, 0.1 μ g PD-135,158 or saline revealed a significant main effect of Week ($F_{(4,112)}=3.533$, $p<.05$) and a significant Week x Drug interaction ($F_{(12,112)}=2.475$, $p<.05$), but failed to reveal a significant main effect of Drug ($F_{(3,28)}=.248$, $p>.05$) during the initial 5 minute test block. Simple main effect analysis and REGW-F multiple

comparisons ($\alpha=0.05$) identified a significant Drug effect 3 weeks post drug administration ($F_{(3,28)}=2.877$, $p<.05$). In particular, mice in the saline condition groomed more frequently in the first 5 minutes of the test period relative to mice which were administered 0.001 μg , 0.01 μg or 0.1 μg PD-135,158 immediately prior to initial apparatus exposure (see Figure 3).

Insert Figure 3 about here

Time in Open Corridors

An analysis of variance of time spent in the open corridors among CD-1 mice challenged with 0.001 μg , 0.01 μg , 0.1 μg PD-135,158 or vehicle revealed a significant main effect of Week ($F_{(4,112)}=19.894$ $p<.05$) and a significant Week x Drug interaction ($F_{(12,112)}=1.932$, $p<.05$), but failed to reveal a significant main effect of Drug ($F_{(3,28)}=0.183$, $p>.05$) for the initial 5 minutes trial block. Simple main effect analysis and subsequent REGW-F multiple comparisons ($\alpha=0.05$) identified a significant Drug effect 6 weeks post drug administration ($F_{(3,28)}=4.456$, $p<.05$). In particular, although the respective CCK-B antagonist doses failed to differentiate mice relative to saline treatment, CD-1 mice which previously received 0.001 μg PD-135,158 were less active than mice which had received either 0.01 μg or 0.1 μg PD-135,158 (see Figure 4).

Insert Figure 4 about here

Corridor Rearing

An analysis of variance of corridor rearing among CD-1 mice challenged with 0.001 µg, 0.01 µg, 0.1 µg PD-135,158 or saline revealed a significant main effect of Week ($F_{(4,112)}=7.698$, $p<.05$) and a significant Week x Drug interaction ($F_{(12,112)}=2.503$, $p<.05$), but failed to reveal a significant main effect of Drug ($F_{(3,28)}=0.207$, $p>.05$) during the initial 5 minute trial block. Simple effect analysis and REGW-F multiple comparisons ($\alpha=0.05$) identified a significant Drug effect 6 weeks post drug administration ($F_{(3,28)}=5.35$, $p<.05$). In particular, CD-1 mice which had initially received 0.001 µg PD-135,158 demonstrated significantly less open corridor rearing than mice initially administered either 0.01 µg or 0.1 µg PD-135,158. The CCK_B antagonist doses failed to differentiate mice from animals in the saline condition (see Figure 5).

Insert Figure 5 about here

Dark Chamber Stretch Attend Postures

An analysis of variance of the stretch attend postures from the dark chambers among CD-1 mice challenged with 0.001 µg, 0.01 µg, 0.1 µg PD-135,158 or saline revealed a significant main effect of Week ($F_{(4,112)}=2.68$, $p<.05$) and a significant Week x Drug interaction ($F_{(12,112)}=2.991$, $p<.05$), but failed to reveal a significant main effect of Drug ($F_{(3,28)}=0.664$, $p>.05$) for the cumulative 10 minutes of the test period. Simple main effect analysis and REGW-F multiple comparisons ($\alpha=0.05$) identified a significant Drug

effect 6 weeks post pharmacological challenge ($F_{(3,28)}=3.389$, $p<.05$). CD-1 mice administered 0.001 μg PD-135,158 on the Immediate test day exhibited more frequent dark chamber stretch attend postures relative to mice in the remaining drug conditions (see Figure 6).

Insert Figure 6 about here

Illuminated Corridor Stretch Attend Postures

An analysis of variance of the stretch attend postures in the illuminated corridors among CD-1 mice challenged with 0.001 μg , 0.01 μg , 0.1 μg PD-135,158 or saline revealed a significant main effect of Week ($F_{(4,112)}=16.092$, $p<.05$), but failed to reveal a significant Drug ($F_{(3,28)}=.486$, $p>.05$) effect or a significant Week x Drug interaction ($F_{(12,112)}=1.406$, $p>.05$) for the first 5 minutes of the test period. Main effects analysis ($\alpha=0.05$) revealed that CD-1 mice performed more stretch attend postures on the Immediate day than those evident on the ensuing behavioural test days. The frequency of illuminated corridor stretch attend postures among CD-1 mice declined significantly during the 3, 6, 9 and 12 week behavioural evaluation intervals (see Figure 7).

Insert Figure 7 about here

Corridor Transition Frequency

An analysis of variance of the corridor transition frequencies among CD-1 mice challenged with 0.001 µg, 0.01 µg, 0.1 µg PD-135,158 or saline revealed a significant main effect of Week ($F_{(4,112)}=8.592$, $p<.05$), but failed to reveal a significant Drug ($F_{(3,28)}=0.666$, $p>.05$) effect or a significant Week x Drug interaction ($F_{(12,112)}=1.47$, $p=>.05$) for the first 5 minutes of the test period. Main effects analysis ($\alpha=0.05$) revealed that CD-1 mice performed significantly more corridor transitions on the Immediate and the 3 week behavioural evaluation intervals relative to mice subsequently tested 6, 9 and 12 weeks post drug administration (see Figure 8).

 Insert Figure 8 about here

Latency to Dark Chamber Entry

An analysis of variance of latency to dark chamber entry among CD-1 mice challenged with 0.001 µg, 0.01 µg, 0.1 µg PD-135,158 or saline revealed a significant main effect of Week ($F_{(4,112)}=10.187$, $p<.05$), but failed to reveal a significant Drug ($F_{(3,28)}=0.719$, $p>.05$) effect or a significant Week x Drug interaction ($F_{(12,112)}=0.81$, $p>.05$). Main effects analysis ($\alpha=0.05$) revealed that latency to dark chamber entry was significantly extended on Immediate behavioural evaluation relative to mice evaluated 3, 6, 9 and 12 weeks post drug administration (see Figure 9).

 Insert Figure 9 about here

Corridor Transition Latency

An analysis of variance of illuminated corridor transition latency among CD-1 mice challenged with 0.001 µg, 0.01 µg, 0.1 µg PD-135,158 or saline revealed a significant main effect of Week ($F_{(4,112)}=3.393$, $p<.05$), but failed to reveal a significant Drug ($F_{(3,28)}=0.518$, $p>.05$) effect or a significant Week x Drug interaction ($F_{(12,112)}=0.81$, $p>.05$). Main effects analysis ($\alpha=0.05$) revealed that CD-1 mice demonstrated significantly reduced latencies to initially switch corridors 3 and 6 weeks post drug administration relative to the Immediate as well as the 9 and 12 week post pharmacological challenge intervals (see Figure 10).

 Insert Figure 10 about here

Corridor Re-Entry Latency

An analysis of variance of corridor re-entry latency among CD-1 mice challenged with 0.001 µg, 0.01 µg, 0.1 µg PD-135,158 or saline failed to reveal significant main effects of Week ($F_{(4,112)}=2.84$, $p>.05$) or Drug ($F_{(3,26)}=.911$, $p>.05$), or a significant Week x Drug interaction ($F_{(12,112)}=.782$, $p>.05$).

Dark Chamber Re-Entry Latency

An analysis of variance of dark chamber re-entry latency among CD-1 mice challenged with 0.001 µg, 0.01 µg, 0.1 µg PD-135,158 or saline revealed a significant main effect of Week ($F_{(4,112)}=5.468$, $p<.05$), but failed to reveal a significant Drug ($F_{(3,28)}=0.455$, $p>.05$) effect or a significant Week x Drug interaction ($F_{(12,112)}=0.74$, $p>.05$). Main effects analysis ($\alpha=0.05$) revealed that CD-1 mice, regardless of pharmacological challenge, re-entered the dark chamber of the apparatus with a significantly reduced latency relative to the remaining behavioural evaluation intervals (see Figure 11).

Insert Figure 11 about here

Figure 2. Mean ($\pm$ SEM) activity scores during the first five minutes of a ten minute behavioural test of CD-1 mice treated intraventricularly with saline, 0.001 μ g, 0.01 μ g or 0.1 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test day followed by sham injection on subsequent tests and exposure to the modified light/dark transition apparatus 10 minutes following injection.

Figure 3. Mean ($\pm$ SEM) grooming scores during the first five minutes of a ten minute behavioural test of CD-1 mice treated intraventricularly with saline, 0.001 μ g, 0.01 μ g or 0.1 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test day followed by sham injection on ensuing tests and exposure to the modified light/dark transition apparatus 10 minutes following injection.

Figure 4. Mean ($\pm$ SEM) time in open during the first five minutes of a ten minute behavioural test of CD-1 mice treated intraventricularly with saline, 0.001 μ g, 0.01 μ g or 0.1 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test day followed by sham injection on subsequent behavioural evaluations and exposed to the modified light/dark transition apparatus 10 minutes following injection.

Figure 5. Mean ($\pm$ SEM) corridor rearing during the first five minutes of a ten minute behavioural test of CD-1 mice treated intraventricularly with saline, 0.001 μ g, 0.01 μ g or 0.1 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test day followed by sham injection on subsequent evaluations and exposure to the modified light/dark transition task 10 minutes following injection.

Figure 6. Mean ($\pm$ SEM) dark chamber stretch attend postures during a ten minute behavioural test of CD-1 mice treated intraventricularly with saline, 0.001 μ g, 0.01 μ g or 0.1 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test day followed by sham injection on subsequent tests and exposure to the modified light/dark transition apparatus 10 minutes following injection.

Figure 7. Mean ($\pm$ SEM) illuminated corridor stretch attend postures during the first five minutes of a ten minute behavioural test of CD-1 mice treated intraventricularly with saline, 0.001 μ g, 0.01 μ g or 0.1 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test day followed by sham injections on ensuing behavioural evaluations and exposure to the modified light/dark transition task 10 minutes following injection.

Figure 8. Mean ($\pm$ SEM) corridor transition frequency during the first five minutes of a ten minute behavioural test of CD-1 mice treated intraventricularly with saline, 0.001 μ g, 0.01 μ g or 0.1 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test day followed by sham injection on subsequent evaluations and exposure to the modified light/dark transition paradigm 10 minutes following injection.

Figure 9. Mean ($\pm$ SEM) latency to dark chamber entry during a ten minute behavioural test of CD-1 mice treated intraventricularly with saline, 0.001 μ g, 0.01 μ g or 0.1 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test day followed by sham injection on subsequent tests and exposure to the modified light/dark transition test 10 minutes following injection.

Figure 10. Mean ($\pm$ SEM) illuminated corridor transition latency during a ten minute behavioural test of CD-1 mice treated intraventricularly with saline, 0.001 μ g, 0.01 μ g or 0.1 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test day followed by sham injection on ensuing tests and exposure to the modified light/dark transition apparatus 10 minutes following injection.

Figure 11. Mean ($\pm$ SEM) dark chamber re-entry latency during a ten minute behavioural test of CD-1 mice treated intraventricularly with saline, 0.001 μ g, 0.01 μ g or 0.1 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test day followed by sham injections on subsequent behavioural evaluations and exposure to the modified light/dark transition task 10 minutes following injection.

Discussion

It will be recalled that Experiment 1 (a) evaluated the putative anxiolytic effects of intraventricular administration of the CCK_B receptor antagonist PD-135,158 in response to a mild psychological stressor (b) ascertained the minimal intraventricular dose of PD-135,158 effecting behavioural change in a modified infrahuman anxiety paradigm and (c) assessed the post-CCK_B antagonist influence during the ensuing 3 month period on anxiety-like behaviour in the modified light-dark paradigm. It is intriguing that salient intraventricular drug schedules and behavioural assessment intervals were detected among CD-1 mice by several behaviours including activity, grooming, open corridor time, corridor rearing and stretch attend postures from the dark chambers. Indeed, with the exception of grooming episodes, which appeared 3 weeks post-drug administration and differentiated saline from the influence of the CCK_B antagonist, demonstrable behavioural measures initially emerged 6 weeks post-drug administration and were attributable to the lowest challenge dose of PD-135,158 administered intraventricularly. It should be underscored that pharmacological challenge of CD-1 mice ten minutes prior to initial modified anxiety paradigm exposure was restricted to the Immediate test interval. Sham drug administration was instituted on the remaining schedule of behavioural evaluation.

Six weeks post drug administration, the CD-1 mice challenged with the 0.001 µg dose of PD-135,158 exhibited behavioural changes consistent with an operational definition of elevated anxiety in the paradigm, including decreased activity, reduced open corridor occupancy and rearing as well as an increased frequency of stretch attend postures from dark chambers of the apparatus. Some CCK_B antagonists (e.g., PD-

134,308, L-365,260), including PD-135,158, have been shown to effect anxiolytic repercussions following systemic administration in rats (Gracey et al., 2000; Griebel et al., 1997; Matto et al., 1997), mice (Costall et al., 1991; Hughes et al., 1990; Belzung et al., 1994), guinea pigs (Rex, Fink & Marsden, 1994) and marmosets (Costall et al., 1991) in the elevated plus maze, the light/dark box and social interaction paradigms. It is intriguing, however, that one laboratory, employing varying doses of the CCK_B antagonists PD-134,308 and PD-135,158 described bell-shaped behaviour specific dose response curves among mice in the light/dark box paradigm (Costall et al., 1991.). For example, mice subcutaneously challenged with either the higher (10 or 30 mg/kg) or lower doses (.0001 or .001 mg/kg) of PD-134,308 spent less time in the open, exhibited decreased corridor transition frequencies and demonstrated elevated dark chamber entry latencies relative to mice challenged with mid-range doses (.01 or 1 mg/kg). A similar behavioural profile was in evidence among mice challenged with PD-135,158. The anxiogenic response detected by the current Experiment following intraventricular injection of PD-135,158 appears consistent with such a non-linear dose response profile. It should be emphasized that the anxiogenic responses detected following low dose PD-135,158 administration only emerged 6 weeks post CCK-B antagonist administration. Indeed, previous investigations employing PD-135,158 have only focused on the acute behavioural effects of such drug challenge, although at least one investigation identified a duration of behavioural influence (e.g., decreased time in dark, increased latency to dark) that exceeded 12 hours (Costall et al., 1991). Yet, the behavioural influence of intraventricular PD-135,158 administration among mice and indeed of most CCK_B antagonists has not been reported. Lateral ventricular drug administration undoubtedly

diffuses to diverse central sites (e.g., amygdala, nucleus accumbens, locus coeruleus) and influences multiple neurochemical systems (e.g., mesolimbic, nigrostriatal, dorsal noradrenergic) directly or more likely by a neurochemical cascade prompting a behavioural profile which would differ substantially from one in which the CCK-B antagonist was directed at a specific central site. To be sure, the simultaneous activation of neural circuitry associated with arousal/attention/vigilance (e.g., dorsal NE bundle), affective reactivity (e.g., mesolimbic system) and/or stimulus priority (e.g., raphe system) will produce organismic preparedness which extracts stimulus (e.g., apparatus) features at variance with site specific drug challenge which may emphasize more discrete episodes of stimulus responsivity. Indeed, the profile of behavioural change in response to pharmacological challenge, in either case, will vary with the neural imprint provided on initial challenge and perhaps the specific anxiety paradigm under investigation.

It is interesting that the profile of behavioural change effected by PD-135,158 administration 6 weeks following initial drug challenge appears to define a blunting of exploration of a novel environment. To be sure, such a behavioural profile is reminiscent of the data provided by Avgustinovich et al. (2000) among BALB, C57 and CBA mice as well as Thierry et al. (1976) among BALB mice exposed to a novel environment. However, such behavioural change emerged within 15 minutes following introduction of animals to an open field (Thierry et al., 1976; Avgustinovich et al., 2000) or an elevated plus maze paradigm (Avgustinovich et al., 2000) in contrast to appreciable delay in the modified corridor transition paradigm. While the differential time frame defining behavioural responsivity may vary with the strain of mouse examined, it is obvious that the behavioural blunting which followed from the experimental protocols of Thierry et al.

and Avgustinovich et al. emanated from initial stressor severity, while the present Experiment minimized the impact of the initial stressor experience by CCK_B antagonist administration. Indeed, intraventricular administration of PD-135,158 among CD-1 mice in this paradigm may have produced a subtle anxiolytic effect upon Immediate exposure to the apparatus, such that neurochemical systems are not sufficiently activated to form longer term response strategies, resulting in future sensitization to the mild psychological stimulus. Thus, after 6 weeks, CD-1 mice treated with PD-135,158 on the initial exposure may experience the re-test as a greater potential threat relative to saline treated mice.

The emergence of rearing, time in the open and locomotor activity among CD-1 mice initially challenged with the low CCK-B antagonist dose 6 weeks following paradigm exposure have been routinely employed in the elevated plus maze (Zangrossi & File, 1992b; Rodgers et al., 1992) and the light/dark box (Crawley, 1991) in mice. These behaviours have been demonstrated to be responsive to anxiogenic drugs including CCK fragments (Crawley et al., 1981; Derrien et al., 1993; Fekete et al., 1994), beta-carboline (File et al., 1985) and picrotoxin (File & Lister, 1984) as well as anxiolytic benzodiazepine agents (Griebel et al., 1997; Crawley & Goodwin, 1980; Costall et al., 1988) and CCK_B receptor antagonists (Costall et al., 1991; Hughes et al., 1990; Belzung et al., 1994). Such paradigms have also detected variations in infrahuman anxiety in behaviours such as stretch attend postures employing similar pharmacological challenges in mice (Pellow et al., 1985; Moser, 1989; Lee & Rodgers, 1990). In contrast to exploratory indices, stretch attend postures may be characterized as the lack of, or aborted exploration attempts. It is unlikely that these various behavioural indices of

anxiety are subserved by a simple neurochemical mechanism. As will be revealed in ensuing descriptions, the conjoint influence of CCK and DA may figure prominently in defining behavioural change and perhaps long term sensitization following initial stressor exposure in CD-1 mice.

It should be emphasized that salient variations in grooming scores were evident among CD-1 mice initially challenged with PD-135,158 3 weeks post CCK antagonist administration. In effect, the CCK antagonist treated mice exhibited reduced grooming episodes with subsequent encounter with the modified corridor transition paradigm. It is curious that the post-CCK antagonist grooming scores deviated substantially from the emerging temporal pattern of exploration and stretch attend behaviours observed in the corridor paradigm. Although grooming has been associated with satiation and the termination of consummatory acts (e.g., sexual behaviour) (Rodgers et al., 2000; Lee et al., 1998), increased grooming frequency often mirrors increases in anxiety (Espejo, 1997; Fiore et al., 1998). Thus, it appears that the doses of PD-135,158 employed in Experiment 1 are anxiolytic relative to saline challenge 3 weeks post drug administration, including the 0.001 μ g PD-135,158 dose which effects anxiogenic effects 6 weeks following drug challenge. Two tentative arguments may be posited at this juncture. The first of these suggests that the behavioural scores secured in Experiment 1 are spurious. Yet salient features or descriptive parameters in support of such a hypothesis are not readily available. Indeed, CD-1 mice in Experiment demonstrated sufficient homogeneity to preclude outliers. It should be considered that the neurochemical substrate(s) underlying grooming is (are) fundamentally different from that (those) associated with exploration indices and stretch attend responsivity in the modified corridor transition task

following CCK-B antagonist administration. Taken together, firm conclusions concerning this measure and the long term influence of PD-135,158 should be held in abeyance.

In contrast to activity scores, stretch attend and conceivably grooming indices among mature male CD-1 mice other behavioural indices measured in Experiment 1, including the frequency of corridor transitions, latency to dark, latency to initially switch corridors, and the initial safe area re-entry latency scores, are not responsive to manipulation by CCK-B antagonist administration in the modified corridor transition test apparatus. Measures of area transition frequency and latency to dark are commonly used to evaluate anxiety levels in the light/dark box, and have been shown to be sensitive to both anxiogenic (Costall et al., 1988; Crawley, Skolnick & Paul, 1984; Young & Johnston, 1991a,b) and anxiolytic (Crawley & Goodwin, 1980; Onaivi & Martin, 1989; Lopez-Rubalcava et al., 1992) agents, including PD-135,158 (Costall et al., 1991). When these behaviours are examined within the modified corridor transition test however, either CCK does not participate in the induction of these behavioural changes, the dose of the PD-135,158 is inappropriate or perhaps the testing intervals are inadequate to detect variations in these behavioural indices of anxiety. It should be noted at this juncture that unlike other paradigms (e.g., elevated plus maze, light/dark box) re-test exposure did not result in the extinction of any of the behavioural indices measured in Experiment 1, allowing for the evaluation of long-term changes in behaviour.

The emerging profile of behavioural change effected by PD-135,158 administration in the corridor paradigm during the 3-month assessment interval appears, at least on first inspection, consistent with the neural time frame depicted by Post &

Weiss (1995) in delineating long-term behavioural change triggered by a stressor or stressor like experience. Acute synaptic events induced by stressors may eventuate in chronic enduring effects which impact on neuronal sensitivity, induced in part by a cascading gene transcriptional mechanism. Endogenous activation of CCK receptors and second messenger systems may provoke activation of immediate early genes including c-fos, c-jun and FRA. Protein synthesis hours after immediate early gene activation alter regulatory factors and induce morphological modifications intraneuronally. In short, intraventricular administration of 0.001 μ g PD-135,158 immediately prior to the modified corridor transition task alters the initial experience of CD-1 mice such that long term neuronal changes occur, resulting in the emergence of heightened anxiety relative to control animals several weeks following pharmacological challenge.

To conclude, the data of Experiment 1 demonstrate that the modified corridor transition task is sensitive to pharmacological manipulation of long-term behavioural change in response to a mild psychological stressor. These data suggest that the initial expression of anxiety like behaviours among CD-1 mice in the corridor paradigm following intraventricular PD-135,158 administration was absent because the neural systems responsible for behaviours sensitive to manipulation with this paradigm require some incubation period before such behaviours can be expressed. The behaviours measured provide sufficient information to allow for increases or decreases in anxiety level to be detected with no extinction of behaviours upon re-test within the modified corridor transition test apparatus. While an anxiolytic effect of PD-135,158 was detected three weeks following intraventricular administration, the lowest dose, 0.001 μ g PD-

135,158 was identified as affecting behavioural changes in a number of measures and was employed to evaluate the effects of intraregional administration in Experiment 2.

Experiments 2A and B

It will be recalled that Experiment 1 was designed to (a) evaluate the putative anxiolytic effects of intraventricular administration of the CCK_B receptor antagonist PD-135,158 in response to a mild psychological stressor, (b) ascertain the minimally effective anxiolytic doses of PD-135,158 and (c) assess the long term effects of initial pharmacological challenge on operationalized measures of anxiety like behaviour in a modified corridor paradigm. It was expected that intraregional administration of the CCK_B antagonist would provide evidence for variable effects relative to intraventricular administration. It is well documented that various central sites appear to participate in the expression of anxiety (e.g., paraventricular nucleus of the hypothalamus, locus coeruleus, and ventral tegmental area). In addition, some of these central sites have been associated with anticipatory anxiety; others appear to be differentially sensitive to stressor severity (e.g., medial prefrontal cortex), while still others may modify motivational (e.g., nucleus accumbens) or affective components (e.g., amygdala).

In particular, the nucleus accumbens has received extensive consideration owing to the postulated role of this site in integrating information between limbic and motor systems (Mogenson & Yim, 1991). The nucleus accumbens has been associated with locomotor activity (Hamilton & Freeman, 1995), reward (Bozarth, 1991; see Salamone et al., 1997, for review) and anxiety (Derrien et al., 1993). It will be recalled that the

nucleus accumbens contains an appreciable density of CCK_A and CCK_B receptors, with the CCK_B receptors more heavily concentrated in the posterior accumbal area (Hokfelt et al., 1991), which receives prominent CCK innervation from the VTA (Hurd et al., 1992). In vitro, administration of CCK-8US and CCK-4 potentiates the inhibitory actions of apomorphine on mesencephalic DA neurons by virtue of a CCK_B receptor interaction (Hommer et al., 1986). Moreover, pretreatment with the CCK_A receptor antagonist, CR-1409, blocks CCK modulated enhancement of DA receptor sensitivity to the D₂ receptor agonist quinpirole which is not blocked by pre-administration of the CCK_B receptor antagonist, L-365,260 (Kelland et al., 1991). Although the apparently opposing effects of the CCK_A and the CCK_B receptors upon DA interactions within the accumbens have been well demonstrated in vitro, the role of these receptors to behavioural expression of anxiety has not been elucidated. In addition, the amygdala may also provide a putative interface between motivated behaviour and emotional lability. However such integrated responses would be clearly dependent upon learning and memory. In effect, the amygdala is a central site influenced by novelty and repeated encounter with mildly anxiogenic events (Ono et al., 1995; Walker et al., 1997). Interestingly, Benkelfat et al. (1995) demonstrated increased cerebral blood flow to the amygdaloid region following anxiety induction provoked by a bolus injection of CCK-4 among healthy volunteers. At present, there are no investigations that describe the behavioural influence of intracerebral CCK antagonists and the short and long term responsivity of infrahuman subjects to a mild psychological stressor in diverse mesolimbic sites. The nucleus accumbens and the amygdala, mesolimbic sites with demonstrable CCK_B receptor density and distinct behavioural function, were employed in evaluating responsivity of CD-1 mice to a mild

psychological stressor following central administration of minimally effective dose of the CCK_B antagonist identified in Experiment 1.

Experiments 2A and 2B employed the minimally effective dose of the intraventricularly administered anxiolytic identified in Experiment 1, 0.001 µg/µl PD-135,158, to evaluate the behavioural profiles generated by intracerebral injection of this CCK_B receptor antagonist in the nucleus accumbens and the central amygdaloid nucleus.

Methods

Subjects

Thirty-two naive, male CD-1 mice were obtained from Charles River (St. Constant, Quebec) at five weeks of age. Animals were acclimated to the animal facility until an acceptable age and/or weight for surgical manipulation (3 months old or 30 g) was achieved. All mice were housed four per cage and maintained on a 12-hour light-dark cycle (light on 7:00 am until 7:00 PM), with food and water available *ad libitum*.

Stereotactic Surgery

Animals were randomly assigned to either the Experiment 2A or 2B surgical procedure. An inhaleable gas mixture of oxygen and halothane (CDMV) was employed to induce surgical anaesthesia. The mouse was placed in a David Kopf Stereotaxic instrument fitted with a mouse adapter, its scalp cleansed and an anterior to posterior incision made to expose the skull surface. For Experiment 2A, co-ordinates used for the surgical implantation of a 23-gauge cannula into the anterior nucleus accumbens were A.P. +0.6 mm. from Bregma, L. 0.7 mm. from the midline and V. -3.6 mm. from a flat

skull surface. For Experiment 2B, co-ordinates used for the surgical implantation of a 23-gauge cannula into the amygdala were A.P. -1.0 mm. from Bregma, 2.2 mm. lateral from the midline and ventral -5.0 mm. from a flat skull surface. A 30-gauge stylette was employed to increase the patency of the implanted cannula. Following surgery, animals were housed individually, placed on a warm heating pad and provided a dietary supplement (Meritene nutritional supplement in wet crushed food pellets) in a recovery room for a period of one week. Following behavioural recovery, mice were returned to the main animal holding area for approximately 7 days until the commencement of behavioural testing.

Apparatus

The modified light/dark test apparatus was identical to that described in Experiment 1.

Procedure

Following recovery from surgery, animals were randomly assigned to either drug or saline conditions ($n = 8/\text{cell}$). Intracranial administration of $0.001 \mu\text{g}/\mu\text{l}$ PD-135,158 (Research Biochemicals International, Natick, MA) and vehicle (saline) was accomplished employing the identical procedures as described in Experiment 1. All initial and repeated behavioural testing procedures were identical to those described in Experiment 1.

Following completion of the Experiments 2A and B, animals were overdosed with sodium pentobarbital and perfused intracardially with 0.9% saline followed by a 10%

formalin solution. The brains were removed from the skull, blocked, frozen and sectioned at 40 μm . Coronal brain sections were stained with cresyl violet and examined under a microscope to verify cannula placement in either the nucleus accumbens or the amygdala.

Statistical Analysis

Dependent variables measured included the behavioural measures as described in Experiment 1. The independent variables used to evaluate these measures were the between group variable drug dose and brain region of cannula implantation, and the within group variable of week of apparatus exposure. Test measures from the first and second 5 minute blocks as well as the total 10 minute length of the test were analysed individually. For simplicity, only the most pertinent test portion analysis is reported in detail in the results section of Experiment 2. Complete analysis summaries for all test portions are reported in Appendix B. Statistical analysis was completed using SPSS 7.0 analysis of variance repeated measure procedure using an alpha level of 0.05 for tests of the main and interaction effects. A priori comparisons between the drug groups and the saline control condition was made using an alpha level of 0.05 and appropriate post hoc multiple comparisons were evaluated within significant main effects and/or interactions using Bonferroni and Ryan Einot Gabriel & Welsch F (REGW-F) tests. Violations of the sphericity assumption for within-subjects effects were treated by adjustment to critical degrees of freedom using Greenhouse-Geisser and Huynh-Feldt corrections as recorded in Appendix B.

Results

Microscopic examination of the coronal brain sections of mice included in Experiments 2a and 2b confirmed accurate placements within the nucleus accumbens ($N = 16$) (see Figure 12) or the central amygdaloid nucleus ($N = 16$) (see Figure 13) respectively. Cannula placements in the nucleus accumbens among CD-1 mice which intruded on the lateral ventricle ($n = 6$), those which were inaccurately placed in adjacent areas (e.g., caudate, septum) ($n = 10$) or which effected ablation of the nucleus accumbens ($n = 8$) were removed from statistical analysis. Likewise, central amygdaloid cannula placements among CD-1 mice which were inaccurately positioned ($n = 8$), those placements which intruded on or effected damage to the central nucleus ($n = 8$) as well as those in which evidence for severe necrosis was apparent were removed from data analysis.

Activity

An analysis of variance of activity, operationalized by line crossings within the apparatus, among CD-1 mice challenged with 0.001 μg PD-135,158 or saline during the initial 5 minute trial block revealed a significant main effect of Week ($F_{(4,112)}=5.07$, $p < .05$) but failed to reveal significant main effects of Drug ($F_{(1,28)}=2.866$, $p > .05$) or Region ($F_{(1,28)}=1.85$, $p > .05$). There were no detected significant interactions of Drug x Region ($F_{(1,28)}=1.85$, $p > .05$), Week x Drug ($F_{(4,112)}=.522$, $p > .05$) or Week x Region ($F_{(4,112)}=1.174$, $p > .05$) or a Week x Drug x Region ($F_{(4,112)}=.729$, $p > .05$) interaction. Main effects analysis ($\alpha=0.05$) revealed CD-1 mice, regardless of drug administration, exhibited higher activity scores Immediately and 3 weeks post drug administration

relative to behavioural evaluation evidenced 6, 9 and 12 weeks following pharmacological challenge (see Figure 14).

Insert Figure 14 about here

Grooming

An analysis of variance of the grooming scores among CD-1 mice challenged with 0.001 μ g PD-135,158 or saline for the first 5 minutes of the test failed to reveal significant main effects of Week ($F_{(4,112)}=1.152$, $p>.05$), Drug ($F_{(1,28)}=1.366$, $p>.05$) or Region ($F_{(1,28)}=1.366$, $p>.05$), interactions of Drug x Region ($F_{(1,28)}=.683$, $p>.05$), Week x Drug ($F_{(4,112)}=1.787$, $p>.05$) or Week x Region ($F_{(4,112)}=1.082$, $p>.05$) or a Week x Drug x Region interaction ($F_{(4,112)}=.515$, $p>.05$).

Time in Open Corridors

An analysis of variance of time spent in the open corridors among CD-1 mice challenged with 0.001 μ g PD-135,158 or saline for the first 5 minutes of the test revealed significant main effects of Week ($F_{(4,112)}=19.916$, $p<.05$), Drug ($F_{(1,28)}=437.253$, $p<.05$) and Region ($F_{(1,28)}=4.945$, $p<.05$), but failed to reveal a Week x Drug ($F_{(4,112)}=.46$, $p>.05$) interaction. Significant Drug x Region ($F_{(1,28)}=4.739$, $p<.05$) and Week x Region ($F_{(4,112)}=2.755$, $p<.05$) interactions were revealed. No detectable Week x Drug x Region ($F_{(4,112)}=2.755$, $p>.05$) interactions were identified. Simple main effects analysis and REGW-F multiple comparisons ($\alpha=0.05$) demonstrated significant Week x Region

interactions 3 and 6 weeks post drug administration. In particular, CD-1 mice with central amygdaloid cannula implants spent more time in the open corridors relative to mice with cannula implantation in the nucleus accumbens ($F'_{s(1,32)}=4.745$, $p<.05$ respectively) (see Figure 15a).

 Insert Figure 15a about here

Simple main effects analysis and REGW-F multiple comparisons ($\alpha=0.05$) of the Drug x Region interactions revealed that among the CD-1 mice with accumbal implantation, animals which had initially received 0.001 μ g PD-135,158 spent more time in the open corridors than those which had initially received saline ($F_{(1,14)}=8.963$, $p<.05$) (see Figure 15b). Moreover, CD-1 mice with nucleus accumbens cannula implants initially challenged with saline spent more time in open than mice with amygdaloid implantation that also initially received vehicle ($F_{(1,14)}=9.791$, $p<.05$) (see Figure 15c).

 Insert Figures 15b and 15c about here

Corridor Rearing

An analysis of variance of corridor rearing among CD-1 mice challenged with 0.001 μ g PD-135,158 or saline for the cumulative ten minutes of the test period revealed significant main effects of Week ($F_{(4,112)}=12.014$, $p<.05$) and Region ($F_{(1,28)}=5.447$, $p<.05$) but not Drug ($F_{(1,28)}=0.015$, $p>.05$). No significant Week x Drug ($F_{(4,112)}=0.479$,

$p > .05$) or Week $\times$ Region ($F_{(4,112)} = 0.559$, $p > .05$) interactions were detected, but a significant Drug $\times$ Region interaction was revealed ($F_{(1,28)} = 9.337$, $p < .05$). The analyses failed to reveal a significant Week $\times$ Drug $\times$ Region interaction ($F_{(4,112)} = .51$, $p > .05$). Simple effects analysis and REGW-F multiple comparisons ($\alpha = 0.05$) of the Drug $\times$ Region interaction revealed two Drug $\times$ Region interactions. In particular, among CD-1 mice that initially received saline, those mice with amygdaloid implantation reared more in the open corridors relative to mice with accumbal implantation ($F_{(1,14)} = 10.93$, $p < .05$) (see Figure 16a); and CD-1 mice that had initially received 0.001 μ g PD-135,158 with amygdaloid implantation were less anxious than animals that initially received saline ($F_{(1,14)} = 4.911$, $p < .05$) (see Figure 16b).

 Insert Figures 16a and 16b about here

Main effects analysis of Week ($\alpha = 0.05$) revealed that the differences of Week replicated those described in the analysis of activity (see Figure 14). Corridor rearing was highest on the immediate test and the behavioural evaluation 3 weeks following drug administration relative to the frequency of corridor rearing among CD-1 mice during the behavioural evaluations 6, 9 and 12 weeks post drug administration.

Dark Chamber Stretch Attend Postures

An analysis of variance of the stretch attend postures from dark chambers among CD-1 mice challenged with 0.001 μ g PD-135,158 or saline for the first 5 minutes of the test period revealed significant main effects of Week ($F_{(4,112)} = 5.025$, $p < .05$) and Region

($F_{(1,28)}=4.341$, $p<.05$) but not Drug ($F_{(1,28)}=.775$, $p>.05$). No significant Week x Drug ($F_{(4,112)}=.834$, $p>.05$), Drug x Region ($F_{(1,28)}=.304$, $p>.05$), Week x Region ($F_{(4,112)}=1.243$, $p>.05$) or Week x Drug x Region ($F_{(4,112)}=.47$, $p>.05$) interactions were detected. Main effects analysis of Week ($\alpha=0.05$) revealed that stretch attend frequency from the dark chamber among CD-1 mice was highest 3, 6, 9 and 12 weeks post drug administration (see Figure 18a). Main effects analysis ($\alpha=0.05$) of Region revealed that CD-1 mice with a nucleus accumbens cannula exhibited significantly elevated stretch attend behaviour relative to mice with central amygdaloid cannula implant (see Figure 17b).

 Insert Figures 17a and 17b about here

Illuminated Corridor Stretch Attend Postures

An analysis of variance of the stretch attend postures in the illuminated corridors among CD-1 mice challenged with 0.001 μ g PD-135,158 or saline for the first 5 minutes of the test period revealed significant main effects of Week ($F_{(4,112)}=4.876$, $p<.05$), but failed to reveal significant main effects of Region ($F_{(1,28)}=3.793$, $p>.05$) or Drug ($F_{(1,28)}=.002$, $p>.05$). Significant Week x Drug ($F_{(4,112)}=4.948$, $p<.05$) and Week x Region ($F_{(4,112)}=3.482$, $p<.05$) interactions were revealed, but a significant Drug x Region interaction was not detected ($F_{(1,28)}=1.818$, $p>.05$). A significant Week x Drug x Region interaction was revealed ($F_{(4,112)}=4.281$, $p<.05$). Simple main effects analyses and REGW-F multiple comparisons ($\alpha=0.05$) revealed four significant Week x Drug x Region interactions: (i) on Immediate exposure to the apparatus, CD-1 mice with

accumbal implantation and challenged with 0.001 μ g PD-135,158 performed significantly less illuminated corridor stretch attend postures than animals which received vehicle ($F_{(1,14)}=5.727$, $p<.05$) (see Figure 18a); (ii) 9 weeks post drug administration CD-1 mice with amygdaloid implantation initially administered 0.001 μ g PD-135,158 exhibited a higher frequency of stretch attend postures than animals initially administered saline ($F_{(1,14)}=5.645$, $p<.05$) (see Figure 18b); and (iii) 6 ($F_{(1,14)}=7.121$, $p<.05$) and (iv) 12 ($F_{(1,14)}=5.0$, $p<.05$) weeks following saline administration, CD-1 mice with a nucleus accumbens cannula demonstrated a significantly higher frequency of stretch attend behaviour in the illuminated corridors relative to mice with central amygdaloid cannula implants (see Figure 18c).

Insert Figures 18a, 18b and 18c about here

Corridor Transition Frequency

An analysis of variance of the corridor transition frequencies among CD-1 mice challenged with 0.001 μ g PD-135,158 or saline for the first 5 minutes of the test period revealed a significant main effect of Week ($F_{(4,112)}=4.599$, $p<.05$) but failed to reveal significant main effects of Drug ($F_{(1,28)}=1.04$, $p>.05$) or Region ($F_{(1,28)}=1.398$, $p>.05$). No significant Drug x Region ($F_{(1,28)}=3.358$, $p>.05$), Week x Drug ($F_{(4,112)}=1.549$, $p<.05$) Week x Region ($F_{(4,112)}=1.669$, $p>.05$) or Week x Drug x Region ($F_{(4,112)}=1.038$, $p>.05$) interactions were detected. Main effects analyses ($\alpha=0.05$) revealed that CD-1 mice exhibited higher corridor transition frequency Immediately and 3 weeks post drug

administration relative to those measured 6, 9 and 12 weeks following initial pharmacological challenge (see Figure 19).

 Insert Figure 19 about here

Latency to Dark Chamber Entry

An analysis of variance of latency to dark chamber entry among CD-1 mice receiving either 0.001 µg PD-135,158 or vehicle revealed significant main effects of Week ($F_{(4,112)}=34.39$, $p<.05$) and Drug ($F_{(1,28)}=5.484$, $p<.05$) but not of Region ($F_{(1,28)}=1.577$, $p>.05$). No significant Drug x Region ($F_{(1,28)}=.115$, $p>.05$), Week x Drug ($F_{(4,112)}=.554$, $p>.05$) Week x Region ($F_{(4,112)}=1.994$, $p>.05$) or Week x Drug x Region ($F_{(4,112)}=.78$, $p>.05$) interactions were detected. Main effect analysis of Test ($\alpha=0.05$) revealed that latency to dark chamber entry was significantly higher on exposure to the apparatus Immediately following drug administration relative to the remaining behavioural evaluations (see Figure 20a). Main effects analysis of Drug ($\alpha=0.05$) revealed that CD-1 mice which initially received 0.001 µg PD-135,158 had significantly delayed latency to dark chamber entries relative to mice initially administered (see Figure 20b).

 Insert Figures 20a and 20b about here

Corridor Transition Latency

An analysis of variance of illuminated corridor transition latency among CD-1 mice challenged with 0.001 μ g PD-135,158 or saline failed to reveal significant main effects of Week ($F_{(4,112)}=.53$, $p>.05$), Drug ($F_{(1,28)}=.478$, $p>.05$) or Region ($F_{(1,28)}=1.076$, $p>.05$). No significant Drug x Region ($F_{(1,28)}=2.069$, $p>.05$), Week x Drug ($F_{(4,112)}=1.383$, $p>.05$) Week x Region ($F_{(4,112)}=1.709$, $p>.05$) or Week x Drug x Region ($F_{(4,112)}=2.028$, $p>.05$) interactions were detected.

Corridor Re-Entry Latency

An analysis of variance of illuminated corridor re-entry latency among CD-1 mice challenged with 0.001 μ g PD-135,158 or saline failed to reveal significant main effects of Week ($F_{(4,112)}=2.149$, $p>.05$), Drug ($F_{(1,28)}=.888$, $p>.05$) or Region ($F_{(1,28)}=1.567$, $p>.05$). No significant Drug x Region ($F_{(1,28)}=1.788$, $p>.05$), Week x Drug ($F_{(4,112)}=1.532$, $p>.05$) Week x Region ($F_{(4,112)}=1.98$, $p>.05$) or Week x Drug x Region ($F_{(4,112)}=1.378$, $p>.05$) interactions were detected.

Dark Chamber Re-Entry Latency

An analysis of variance of dark chamber re-entry latency among CD-1 mice challenged with 0.001 μ g PD-135,158 or saline failed to reveal significant main effects of Week ($F_{(4,112)}=3.961$, $p>.05$), Drug ($F_{(1,28)}=2.359$, $p>.05$) or Region ($F_{(1,28)}=1.461$, $p>.05$). No significant Drug x Region ($F_{(1,28)}=.000$, $p>.05$), Week x Drug ($F_{(4,112)}=.893$, $p>.05$) Week x Region ($F_{(4,112)}=1.737$, $p>.05$) or Week x Drug x Region ($F_{(4,112)}=.387$, $p>.05$) interactions were detected.

Figure 12. Schematic representation of cannula placements in the nucleus accumbens in CD-1 mice in a rostral-caudal plane.

◆ Saline

● 0.001 μ g PD-135,158

Bregma +1.0 mm

Bregma +0.70 mm

Bregma +0.48 mm

Figure 13. Schematic representation of cannula placements in the central amygdaloid nucleus in CD-1 mice in a rostral-caudal plane.

◆Saline

78

◆0.001 μg PD-135,158

Figure 14. Mean ($\pm$ SEM) activity scores during the first 5 minutes of a 10 minute behavioural test of CD-1 mice treated intracranially with saline or 0.001 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test day followed by sham injection on subsequent tests and exposure to the modified light/dark transition test 10 minutes following injection.

Figure 15a. Mean ($\pm$ SEM) time in open during the first 5 minutes of a 10 minute behavioural test of CD-1 mice treated intracranially with saline or 0.001 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test day followed by sham injection on subsequent evaluations and exposure to the modified light/dark transition test apparatus 10 minutes following injection.

Figure 15b. Mean ($\pm$ SEM) time in open during the first 5 minutes of a 10 minute behavioural test of CD-1 mice treated intracranially with saline or 0.001 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test day followed by sham injection on ensuing tests and exposure to the modified light/dark transition test apparatus 10 minutes following injection.

Figure 15c. Mean ($\pm$ SEM) time in open during the first 5 minutes of a 10 minute behavioural test of CD-1 mice treated intracranially with saline or 0.001 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test day followed by sham injection on subsequent behavioural evaluations and exposure to the modified light/dark transition test apparatus 10 minutes following injection.

Figure 16a. Mean ($\pm$ SEM) corridor rearing during a 10 minute behavioural test of CD-1 mice treated intracranially with saline or 0.001 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test day followed by sham injection on subsequent tests and exposure to the modified light/dark transition test apparatus 10 minutes following injection.

Figure 16b. Mean ($\pm$ SEM) corridor rearing during a 10 minute behavioural test of CD-1 mice treated intracranially with saline or 0.001 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test day followed by sham injection on subsequent tests and exposure to the modified light/dark transition test apparatus 10 minutes following injection.

Figure 17a. Mean ($\pm$ SEM) dark chamber stretch attend postures during the first 5 minutes of a 10 minute behavioural test of CD-1 mice treated intracranially with saline or 0.001 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test days followed by sham injection on ensuing tests and exposed to the modified light/dark transition test apparatus 10 minutes following injection.

Figure 17b. Mean ($\pm$ SEM) dark chamber stretch attend postures during the first 5 minutes of a 10 minute behavioural test of CD-1 mice treated intracranially with saline or 0.001 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test day followed by sham injection on ensuing behavioural tests and exposed to the modified light/dark transition test apparatus 10 minutes following injection.

Figure 18a. Mean ($\pm$ SEM) illuminated corridor stretch attend postures during the first 5 minutes of a 10 minute behavioural test of CD-1 mice treated intracranially with saline or 0.001 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test day followed by sham injection on subsequent tests and exposure to the modified light/dark transition test apparatus 10 minutes following injection.

Figure 18b. Mean ($\pm$ SEM) illuminated corridor stretch attend postures during the first 5 minutes of a 10 minute behavioural test of CD-1 mice treated intracranially with saline or 0.001 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test day followed by sham injection on subsequent tests and exposure to the modified light/dark transition test apparatus 10 minutes following injection.

Figure 18c. Mean ($\pm$ SEM) illuminated corridor stretch attend postures during the first 5 minutes of a 10 minute behavioural test of CD-1 mice treated intracranially with saline or 0.001 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test day followed by sham injection on subsequent tests and exposure to the modified light/dark transition test apparatus 10 minutes following injection.

Figure 19. Mean ($\pm$ SEM) corridor transition frequency during the first 5 minutes of a 10 minute behavioural test of CD-1 mice treated intracranially with saline or 0.001 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test day followed by sham injections on ensuing behavioural evaluation and exposure to the modified light/dark transition test apparatus 10 minutes following injection.

Figure 20a. Mean ($\pm$ SEM) latency to dark chamber entry during a 10 minute behavioural test of CD-1 mice treated intracranially with saline or 0.001 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test day followed by sham injection on subsequent tests and exposure to the modified light/dark transition test apparatus 10 minutes following injection.

Figure 20b. Mean ($\pm$ SEM) latency to dark chamber entry during a 10 minute behavioural test of CD-1 mice treated intracranially with saline or 0.001 μ g of the selective CCK_B receptor antagonist PD-135,158 on the Immediate test day followed by sham injection on subsequent tests and exposure to the modified light/dark transition test apparatus 10 minutes following injection.

Discussion

It is curious that perusal of the literature to date fails to reveal investigations focusing on the anxiolytic influence of intracerebral CCK antagonists and concomitant acute and long-term responsivity of infrahuman subjects to mild psychological stressors in diverse mesolimbic sites. Experiments 2b and 2b employed the minimally effective dose (0.001 $\mu\text{g}/\mu\text{l}$ PD-135,158) of the intraventricularly administered CCK_B receptor antagonist identified in Experiment 1, to evaluate the behavioural profiles generated by intracerebral injection of PD-135,158 in the nucleus accumbens and the central amygdaloid nucleus.

Consistent with the results of Experiment 1, intraregional injection of 0.001 μg PD-135,158 among CD-1 mice promoted behavioural alterations following central amygdaloid and nucleus accumbens administration. In contrast to the anxiogenic influence of intraventricular PD-135,158 administration in the corridor paradigm (i.e., within 6 weeks), CD-1 mice with cannulae in the central amygdaloid nucleus exhibited behavioural change 9 weeks following central injection of PD-135,158. At that time, the anxiogenic effect of PD-135,158 emerged among CD-1 mice as evidenced by an increased frequency of illuminated corridor stretch attend postures relative to vehicle treated mice. Conspicuous differences were also observed among CD-1 mice with cannulas in the nucleus accumbens. In particular, on the Immediate test day, CD-1 mice challenged with PD-135,158 in the nucleus accumbens performed more illuminated corridor stretch attend postures relative to saline treated mice. Nevertheless, this time dependant behaviour indicative of increased anxiety may be contrasted with the overall time in open behaviour among animals with accumbal cannulas. Indeed, CD-1 mice

challenged with PD-135,158 in the nucleus accumbens spent more cumulative time in the open relative to mice administered saline.

In contrast to the detection of dark chamber stretch attend postures observed in Experiment 1 employing 0.001 μ g PD-135,158, Experiment 2 detected differences in illuminated corridor stretch attend postures. It is intriguing that in Experiment 2 significant changes in illuminated corridor stretch attend postures emerged immediately following PD-135,158 administration in CD-1 mice with nucleus accumbens cannulas as compared to 9 weeks post CCK_B antagonist injection in CD-1 mice with amygdaloid implantation. In particular, behavioural changes effected by initial intra-amygdaloid drug administration of 0.001 μ g PD-135,158 in CD-1 mice do not emerge immediately but rather following long term integration of the emotional and behavioural responses to the mild psychological stressor (e.g., modified corridor transition test apparatus) (Ono et al, 1995; Walker et al., 1997).

It is intriguing that some behaviours among CD-1 mice in the modified corridor paradigm were not responsive to PD-135,158 administration following intraregional administration. In particular, consistent with the effects seen using intraventricular injection, amygdaloid injection of PD-135,158 promoted an anxiogenic effect on cumulative rearing scores relative to saline injection rather than emerging 6 weeks post drug challenge as seen with intraventricular administration. Furthermore, intra-accumbal injection of PD-135,158 among CD-1 mice relative to mice receiving vehicle appears to promote an anxiolytic effect when time in the open portions of the modified corridor transition test was examined. Such an effect should be contrasted with the increase in

anxiety as measured by illuminated corridor stretch attend postures that emerges immediately following pharmacological challenge with the CCK_B antagonist.

Consistent with the results of Experiment 1, a number of measured behaviours (e.g., frequency of corridor transitions, various latency measures) in the modified corridor transition task among mature male CD-1 mice were found not to be sensitive to intracranial pharmacological challenge with 0.001 µg PD-135,158. It is of particular interest that some measures (e.g., activity, grooming, dark chamber stretch attend postures) which appeared responsive to intraventricular injection with the CCK_B antagonist did not appear to be receptive to accumbal or amygdaloid administration of PD-135,158 at this dose range within this paradigm, while notable changes in other behaviours (e.g., illuminated corridor stretch attend postures) are detected solely with intraregional administration.

The stressful nature of cannula implantation and surgical manipulation have been previously described (Zacharko et al., 1999) and it is not surprising that differences between CD-1 mice with cannula implantation in the nucleus accumbens relative to mice with central amygdaloid implantation were detected in some of the behavioural indices examined (e.g., time in open, rears). Indeed, ablation of portions of the amygdala has been associated with the loss of the affective component of events or objects (Gallagher & Chiba, 1996; Scott et al., 1997), and in particular rats with lesions of the central amygdaloid nucleus exhibit reductions in the suppression of behaviours elicited by a conditioned fear stimulus but retained ability to direct their actions to avoid further presentation of the fearful stimulus (Killcross et al., 1997). Thus the apparently less anxious state of CD-1 mice with central amygdaloid cannulas may be attributable to

increased sensitivity to surgical procedure relative to mice with nucleus accumbens cannulas. While this difference between some control groups necessarily restricts the scope of possible inter-regional conclusions to be drawn from Experiment 2, it should not overshadow certain significant differences revealed by the analysis that are not affected by comparisons between the nucleus accumbens and the amygdala.

In summary, the results of Experiment 2 were consistent with those of Experiment 1. With one exception, the 0.001 μg dose of PD-135,158 was anxiogenic upon intraregional administration, although this anxiogenic effect could be seen initially as well as up to nine weeks post drug administration. Behavioural differences appeared from injections within the nucleus accumbens earlier than within the amygdala, which is consistent with the proposal that initial mild psychological stressors are immediately encoded by the nucleus accumbens and subsequently interpreted and transformed by amygdaloid processes.

Conclusions

Current animal models of anxiety permit the immediate evaluation of putative anxiolytic agents. However protracted evaluation of behavioural profiles in infrahuman subjects following repeated exposure to relatively mild stressors, which may more parsimoniously mirror the development of human psychopathology including panic, is precluded in the elevated plus maze and the light/dark paradigms. Yet stressor exposure promotes long-term changes in CCK receptor density and sensitivity in various brain areas including the hypothalamus of mice and rats (Hink et al., 1995) and the rat

hippocampus (Harro et al., 1995). Such protracted neural events may promote enduring behavioural changes that favour progression of panic and depression. The modified corridor transition test apparatus appears to favour empirical evaluation of long-term behavioural change influenced by central CCK_B antagonist administration.

Although PD-135-158 promotes an anxiolytic influence in some animal models of anxiety (Costall et al., 1991; Hughes et al., 1990; Belzung et al., 1994), the minimally behaviourally effective dose used in this thesis prompted an anxiogenic effect. In view of the non-linear dose response curves of some pharmacological agents which influence central CCK activity (Costall et al., 1991) it appears of advantage to determine the circumstances associated with the induction of anxiogenic effects promoted by PD-135,158 and conceivably any putative anxiolytic consequences of PD-135,158 among CD-1 mice in the modified corridor paradigm. In contrast, intra-accumbal administration of PD-135,158 resulted in an immediate anxiogenic effect followed by a cumulative anxiolytic effect. It would be of interest to ascertain the effects of CCK antagonists within other mesolimbic regions (e.g., ventral tegmental area) and subregions (e.g., amygdaloid sub-nuclei). In particular, when selective CCK_A receptor antagonists become available, the interplay between A and B receptor subtypes within the core and the shell portions of the nucleus accumbens may provide some insight into the opposing influences of PD-135,158 administration into the accumbens.

The ability of intravenous administration of CCK-4 in human volunteers in clinical settings to provoke behavioural effects reminiscent of panic attacks (Bradwejn & Koszycki, 1990; Benkelfat et al., 1995) has caused the promotion of CCK receptor antagonists as potential anti-panic and anti-anxiety treatments (Bradwejn & Koszycki,

1994, Bradwejn et al., 1994). However, the results of infrahuman research into the anxiolytic potential of such agents, particularly CCK_B antagonists, remain ambiguous (Pande et al., 1999). In particular, the influence of such agents has been difficult to demonstrate using animal models of anxiety (e.g., elevated plus maze) that are sensitive to the anxiolytic effects of other agents (e.g., benzodiazepines). Animal models of anxiety other than those primarily sensitive to benzodiazepines must be utilized in order for the putative anxiolytic potential of agents such as CCK_B receptor antagonists to be ascertained.

In conclusion, the modified corridor transition test apparatus employed in this thesis appears to allow evaluation of long-term behavioural change as influenced by intracranial CCK_B antagonist administration. Pharmacological challenge with 0.001 µg PD-135,158 increases anxiety level when administered intraventricularly or in the amygdala, yet behavioural measures reflecting changes in anxiety emerge at different rates dependent upon the region of PD-135,158 administration in CD-1 mice. Intra-accumbal administration of 0.001 µg PD-135,158 among CD-1 mice has immediate anxiogenic effects followed by cumulative decreases in anxiety levels.

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Appendix A - ANOVA Tables**Experiment #1**

Experiment 1 - ANOVA Tables for Intraventricular Administration

Activity

First 5 Minutes of Test

Between Group Effects

Effect	Statistic	F	DF	P
Drug	SS=13455.819 MS=4485.273	0.716	3	0.000
Error	SS=175449.6 MS=6266.056		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=74576.413 MS=18644.103 G-G Adj DF H-F Adj DF	12.167	4 3.056 3.84	0.000 0.000 0.000
Week*Drug	SS=48208.337 MS=4017.361 G-G Adj DF H-F Adj DF	2.622	12 9.168 11.52	0.004 0.022 0.053
Error	SS=171620.1 MS=1532.322 G-G Adj DF H-F Adj DF		112 85.568 107.52	

Second 5 Minutes of Test

Between Group Effects

Effect	Statistic	F	DF	P
Drug	SS=9709.925 MS=3236.642	0.844	3	0.481
Error	SS=107390.7 MS=3835.380		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=6276.913 MS=1569.228	1.536	4	0.196
Week*Drug	SS=13667.888 MS=1138.991	1.115	12	0.355
Error	SS=114389.6 MS=1021.336		112	

Total 10 Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=47929.350 MS=15976.45	0.842	3	0.000
Error	SS=531273.0 MS=18974.038		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=91874.6 MS=22968.65	5.945	4	0.000
Week*Drug	SS=101183.4 MS=8431.95	2.183	12	0.017
Error	SS=432687.2 MS=3863.279		112	

Grooming**First 5 Minutes of Test****Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=0.65 MS=0.217	0.248	3	0.862
Error	SS=24.45 MS=0.873		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=6.75	3.533	4	0.011
	MS=1.687			
	G-G Adj DF		2.792	0.028
	H-F Adj DF		3.464	0.01
Week*Drug	SS=14.6	2.475	12	0.007
	MS=1.217			
	G-G Adj DF		8.376	0.008
	H-F Adj DF		10.392	0.005
Error	SS=55.05		112	
	MS=0.492			
	G-G Adj DF		78.176	
	H-F Adj DF		96.992	

Second 5 Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=2.075 MS=.692	0.725	3	0.545
Error	SS=26.7 MS=.954		28	

Between Group Effects

Effect	Statistic	F	DF	P
Week	SS=19.35	5.348	4	0.001
	MS=4.838			
	G-G Adj DF		2.52	0.006
	H-F Adj DF		3.084	0.002
Week*Drug	SS=12.55	1.156	12	0.323
	MS=1.046			
	G-G Adj DF		7.56	0.313
	H-F Adj DF		9.252	0.284
Error	SS=101.3		112	
	MS=0.904			
	G-G Adj DF		70.56	
	H-F Adj DF		86.352	

Total 10 Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=3.025 MS=1.008	0.386	3	0.764
Error	SS=73.15 MS=2.613		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=48.25 MS=12.062 G-G Adj DF H-F Adj DF	6.187	4 2.72 3.364	0.000 0.002 0.000
Week*Drug	SS=28.6 MS=2.383 G-G Adj DF H-F Adj DF	1.223	12 8.16 10.092	0.277 0.206 0.211

Time in Open Corridors**First 5 Minutes of Test****Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=4570.569 MS=1523.523	0.183	3	0.907
Error	SS=232503.1 MS=8308.683		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=195464.3 MS=48866.069	19.894	4	0.000
Week*Drug	SS=56944.775 MS=4745.398	1.932	12	0.038
Error	SS=275107.8 MS=2456.319		112	

Second 5 Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=5140.019 MS=1713.34	0.894	3	0.894
Error	SS=237688.4 MS=8488.871		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=50043.537 MS=12510.884 G-G Adj DF H-F Adj DF	5.366	4 2.92 3.644	0.001 0.002 0.000
Week*Drug	SS=25393.263 MS=2116.105 G-G Adj DF H-H Adj DF	0.908	12 8.76 10.932	0.542 0.347 0.366
Error	SS=261132.0 MS=2331.536 G-G Adj DF H-F Adj DF		112 81.76 102.032	

Total 10 Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=10970.25 MS=3656.75	0.12	3	0.948
Error	SS=853881.3 MS=30495.759		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=354193.3	12.131	4	0.000
	MS=88548.322			
	G-G Adj DF		3.048	0.000
	H-F Adj DF		3.828	0.000
Week*Drug	SS=134684.1	1.538	12	0.121
	MS=11223.672			
	G-G Adj DF		9.144	0.139
	H-F Adj DF		11.484	0.107
Error	SS=817524.3		112	
	MS=7299.324			
	G-G Adj DF		85.344	
	H-F Adj DF		107.184	

Corridor Rearing**First 5 Minutes of Test****Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=276.85	0.207	3	0.891
	MS=92.283			
Error	SS=12485.45		28	
	MS=445.909			

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=4021.35	7.698	4	0.000
	MS=1005.338			
Week*Drug	SS=3922.15	2.503	12	0.006
	MS=326.846			
Error	SS=14627.3		112	
	MS=130.601			

Second 5 Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=974.65 MS=324.883	0.773	3	0.519
Error	SS=11762.55 MS=420.091		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=1306.812 MS=326.703 G-G Adj DF H-F Adj DF	2.837	4 2.912 3.636	0.028 0.048 0.02
Week*Drug	SS=971.287 MS=80.941 G-G Adj DF H-F Adj DF	0.703	12 8.736 10.908	0.746 0.527 0.568
Error	SS=12898.7 MS=115.167 G-G Adj DF H-F Adj DF		112 81.536 101.808	

Total 10 Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=2064.059 MS=688.023	0.447	3	0.722
Error	SS=43144.525 MS=1540.876		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=8835.1 MS=2208.775	5.616	4	0.000
Week*Drug	SS=6104.4 MS=508.7	1.293	12	0.232
Error	SS=44050.1 MS=393.304		112	

Dark Chamber Stretch Attend Postures**First 5 Minutes of Test****Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=49.874 MS=16.625	0.791	3	0.000
Error	SS=588.285 MS=21.01		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=276.963 MS=69.241 G-G Adj DF H-F Adj Df	1.639	4 2.112 2.536	0.169 0.335 0.254
Week*Drug	SS=1314.288 MS=109.524 G-G Adj DF H-F Adj DF	2.592	12 6.336 7.608	0.004 0.011 0.012
Error	SS=4731.95 MS=42.25 G-G Adj DF H-F Adj DF		112 59.136 71.008	0.004 0.011 0.012

Second 5 Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=133.719 MS=44.573	1.225	3	0.319
Error	SS=1018.925 MS=36.39		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=138.75	2.875	4	0.026
	MS=34.688			
	G-G Adj DF		2.868	0.048
	H-F Adj DF		3.576	0.02
Week*Drug	SS=303.25	2.095	12	0.023
	MS=25.271			
	G-G Adj DF		8.604	0.019
	H-F Adj DF		10.728	0.013
Error	SS=1351.2		112	0.023
	MS=12.064			
	G-G Adj DF		80.304	0.019
	H-F Adj DF		100.128	0.013

Total 10 Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=464.15 MS=154.717	0.664	3	0.581
Error	SS=6528.55 MS=233.162		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=731.788	2.68	4	0.035
	MS=182.947			
	G-G Adj DF		2.656	0.077
	H-F Adj DF		3.276	0.038
Week*Drug	SS=2449.663	2.991	12	0.001
	MS=204.139			
	G-G Adj DF		7.968	0.003
	H-F Adj DF		9.828	0.002
Error	SS=7644.95		112	
	MS=68.258			
	G-G Adj DF		74.368	
	H-F Adj DF		91.728	

Illuminated Corridor Stretch Attend Postures**First 5 Minutes of Test****Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=7.819 MS=2.606	0.486	3	0.695
Error	SS=150.225 MS=5.365		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=152.963 MS=38.241 G-G Adj DF H-F Adj DF	16.092	4 2.504 3.068	0.000 0.000 0.000
Week*Drug	SS=40.088 MS=3.341 G-G Adj DF H-F Adj DF	1.406	12 7.512 9.204	0.174 0.177 0.184
Error	SS=266.15 MS=2.376 G-G Adj DF H-F Adj DF		112 70.112 85.904	0.174 0.177 0.184

Second 5 Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=0.750 MS=0.25	0.517	3	0.674
Error	SS=13.55 MS=0.484		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=8.35	3.949	4	0.005
	MS=2.088			
	G-G Adj DF		1.532	0.126
	H-F Adj DF		1.776	0.085
Week*Drug	SS=9.25	1.458	12	0.151
	MS=0.771			
	G-G Adj DF		4.596	0.098
	H-F Adj DF		5.328	0.19
Error	SS=59.2		112	
	MS=0.529			
	G-G Adj DF		42.896	
	H-F Adj DF		49.728	

Total Ten Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=10.569 MS=3.523	0.55149	3	0.668
Error	SS=187.375 MS=6.692		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=174.475	14.782	4	0.000
	MS=43.619			
	G-G Adj DF		2.588	0.000
	H-F Adj DF		3.18	0.000
Week*Drug	SS=53.025	1.497	12	0.135
	MS=4.419			
	G-G Adj DF		7.764	0.131
	H-F Adj DF		9.54	0.131
Error	SS=330.5		112	
	MS=2.951			
	G-G Adj DF		72.464	
	H-F Adj DF		89.04	

Corridor Transition Frequency**First 5 Minutes of Test****Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=255.719 MS=85.24	0.666	3	0.58
Error	SS=3585.775 MS=128.063		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=1119.838 MS=279.959 G-G Adj DF H-F Adj DF	8.592	4 3.012 3.776	0.000 0.000 0.000
Week*Drug	SS=574.813 MS=47.901 G-G Adj DF H-F Adj DF	1.47	12 9.036 11.328	0.146 0.169 0.137
Error	SS=3649.35 MS=32.583 G-G Adj DF H-F Adj DF		112 84.336 105.728	

Second 5 Minutes of Test**Between Group Effects**

Effects	Statistic	F	DF	P
Drug	SS=113.525 MS=37.842	0.683	3	0.57
Error	SS=1550.45 MS=55.373		28	

Within Group Effects

Effects	Statistic	F	DF	P
Week	SS=182.662 MS=45.666	2.625	4	0.038
Week*Drug	SS=353.287 MS=29.441	1.693	12	0.078
Error	SS=1948.05 MS=17.393		112	

Total 10 Minutes of Test**Between Group Effects**

Effects	Statistic	F	DF	P
Drug	SS=696.219 MS=232.073	0.707	3	0.556
Error	SS=9196.725 MS=328.454		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=1539.1 MS=384.775 G-G Adj DF H-F Adj DF	5.298	4 2.9 3.616	0.001 0.003 0.001
Week*Drug	SS=1491.25 MS=124.271 G-G Adj DF H-F Adj DF	1.711	12 8.7 10.848	0.073 0.05 0.04
Error	SS=8133.65 MS=72.622 G-G Adj DF H-F Adj DF		112 81.2 101.248	0.073 0.05 0.04

Latency to Dark Chamber Entry**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=505.074 MS=168.358	0.719	3	0.074
Error	SS=6320.668 MS=234.099		27	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=5004.173 MS=1251.043 G-G Adj DF H-F Adj DF	10.187	4 1.3 1.492	0.000 0.01 0.005
Week*Drug	SS=1193.3 MS=99.442 G-G Adj DF H-F Adj DF	0.81	12 3.9 4.476	0.64 0.381 0.319
Error	SS=12363.636 MS=122.802 G-G Adj DF H-F Adj DF		108 35.1 40.284	

Initial Corridor Transition Latency**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=5912.266 MS=1970.755	0.518	3	0.674
Error	SS=95158.727 MS=3806.349		25	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=17542.133	3.393	4	0.012
	MS=1898.879			
	G-G Adj DF		2.192	0.071
	H-F Adj DF		2.7	0.034
Week*Drug	SS=22786.543	1.469	12	0.148
	MS=1292.36			
	G-G Adj DF		6.576	0.103
	H-F Adj DF		8.1	0.123
Error	SS=129236.0		100	0.148
	MS=1292.36			
			54.8	0.103
			67.5	0.123

Initial Corridor Re-Entry Latency**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=2313.49 MS=771.163	0.911	3	0.449
Error	SS=22003.904 MS=846.304		26	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=8972.911	2.84	4	0.028
	MS=2243.228			
	G-G Adj DF		1.824	0.173
	H-F Adj DF		2.18	0.114
Week*Drug	SS=7409.864	0.782	12	0.668
	MS=617.489			
	G-G Adj DF		5.472	0.517
	H-F Adj DF		6.54	0.412
Error	SS=82141.043		112	
	MS=789.827			
	G-G Adj DF		51.072	
	H-F Adj DF		61.04	

Dark Chamber Re-Entry Latency**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=147.101 MS=49.034	0.455	3	0.716
Error	SS=2800.132 MS=107.697		26	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=1388.761 MS=347.19 G-G Adj DF H-F Adj DF	5.468	4 1.884 2.26	0.000 0.023 0.01
Week*Drug	SS=563.676 MS=46.973 G-G Adj DF H-F Adj DF	0.74	12 5.562 6.78	0.71 0.525 0.423
Error	SS=6603.457 MS=63.495 G-G Adj DF H-F Adj DF		112 52.752 63.28	

Appendix B - ANOVA Tables**Experiment 2**

Experiment 2- ANOVA Tables for Intraregional Administration**Activity****First 5 Minutes of Test****Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=18684.006 MS=18684.006	2.866	1	0.102
Region	SS=12058.256 MS=12058.256	1.85	1	0.185
Drug*Region	SS=15307.656 MS=15307.656	2.348	1	0.137
Error	SS=182511.3 MS=6518.262		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=47476.150 MS=11869.038	5.069	4	0.001
Week*Drug	SS=4890.025 MS=1222.506	0.522	4	0.72
Week*Region	SS=10994.65 MS=2748.663	1.174	4	0.326
Week*Drug*Region	SS=6831.125 MS=1707.781	0.729	4	0.574
Error	SS=326851.6 MS=1945.545		112	

Second 5 Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=1123.6 MS=1123.6	0.351	1	0.558
Region	SS=2.5 MS=2.5	0.001	1	0.978
Drug*Region	SS=0.9 MS=0.9	0.000	1	0.987
Error	SS=89607.4 MS=3200.264		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=7472.838	1.539	4	0.196
	MS=1868.209			
	G-G Adj DF		2.924	0.221
	H-F Adj DF		3.656	0.138
Week*Drug	SS=3679.588	0.758	4	0.555
	MS=919.897			
	G-G Adj DF		2.924	0.532
	H-F Adj DF		3.656	0.432
Week*Region	SS=3051.313	0.628	4	0.643
	MS=762.828			
	G-G Adj DF		2.924	0.609
	H-F Adj DF		3.656	0.516
Week*Drug*Region	SS=7836.913	1.614	4	0.176
	MS=1959.228			
	G-G Adj DF		2.924	0.202
	H-F Adj DF		3.656	0.124
Error	SS=135949.4		112	
	MS=1213.833			
	G-G Adj DF		81.872	
	H-F Adj DF		102.368	

Total 10 Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=6225.025 MS=6225.025	0.427	1	0.519
Region	SS=8584.9 MS=8584.9	0.589	2	0.449
Drug*Region	SS=15288.1 MS=15288.1	1.049	1	0.314
Error	SS=407914.1 MS=14568.363		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=112877.3	5.576	4	0.000
	MS=28219.328			
	G-G Adj DF		2.892	0.002
	H-F Adj DF		3.608	0.000
Week*Drug	SS=19256.913	0.951	4	0.437
	MS=4814.228			
	G-G Adj DF		2.892	0.437
	H-F Adj DF		3.608	0.335
Week*Region	SS=13488.213	0.775	4	0.544
	MS=3372.053			
	G-G Adj DF		2.892	0.527
	H-F Adj DF		3.608	0.428
Week*Drug*Region	SS=13488.213	0.666	4	0.617
	MS=3372.053			
	G-G Adj DF		2.892	0.59
	H-F Adj DF		3.608	0.496
Error	SS=566838.6		112	
	MS=5061.059			
	G-G Adj DF		80.976	
	H-F Adj DF		101.024	

Grooming**First 5 Minutes of Test****Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=10.506	1.366	1	0.252
	MS=10.506			
Region	SS=5.256	1.366	1	0.415
	MS=5.256			
Drug*Region	SS=0.156	0.683	1	0.888
	MS=0.156			
Error	SS=215.425		28	
	MS=7.694			

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=10.212 MS=2.553 G-G Adj DF H-F Adj DF	1.152	4 2.184 2.628	0.336
Week*Drug	SS=15.838 MS=3.959 G-G Adj DF H-F Adj DF	1.787	4 2.184 2.628	0.136
Week*Region	SS=9.588 MS=2.397 G-G Adj DF H-F Adj DF	1.082	4 2.184 2.628	0.369
Week*Drug*Region	SS=4.562 MS=1.141 G-G Adj DF H-F Adj DF	0.515	4 2.184 2.628	0.725
Error	SS=248.2 MS=2.216 G-G Adj DF H-F Adj DF		112 61.152 73.584	

Second 5 Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=2.256 MS=2.256	0.594	1	0.447
Region	SS=0.306 MS=0.306	0.081	1	0.779
Drug*Region	SS=0.0562 MS=0.0562	0.015	1	0.904
Error	SS=106.325 MS=3.797		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=11.65	2.156	4	0.079
	MS=2.912			
	G-G Adj DF		2.368	0.175
	H-F Adj DF		2.88	0.111
Week*Drug	SS=5.525	1.022	4	0.399
	MS=1.381			
	G-G Adj DF		2.368	0.495
	H-F Adj DF		2.88	0.272
Week*Region	SS=5.474	1.013	4	0.404
	MS=1.369			
	G-G Adj DF		2.368	0.364
	H-F Adj DF		2.88	0.276
Week*Drug*Region	SS=0.85	0.157	4	0.959
	MS=0.213			
	G-G Adj DF		2.368	0.918
	H-F Adj DF		2.88	0.893
Error	SS=151.3		112	
	MS=1.351			
	G-G Adj DF		66.304	
	H-F Adj DF		80.64	

Total 10 Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=21.025	1.0	1	0.326
	MS=21.025			
Region	SS=7.225	0.343	2	0.563
	MS=7.225			
Drug*Region	SS=0.00	0.000	1	1.0
	MS=0.00			
Error	SS=588.95		28	
	MS=21.034			

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=41.062	1.857	4	0.123
	MS=10.266			
	G-G Adj DF		2.136	0.276
	H-F Adj DF		2.564	0.2
Week*Drug	SS=32.912	1.488	4	0.211
	MS=8.228			
	G-G Adj DF		2.136	0.373
	H-F Adj DF		2.564	0.291
Week*Region	SS=26.338	1.191	4	0.319
	MS=6.584			
	G-G Adj DF		2.136	0.473
	H-F Adj DF		2.564	0.39
Week*Drug*Region	SS=7.188	0.325	4	0.861
	MS=1.797			
	G-G Adj DF		2.136	0.874
	H-F Adj DF		2.564	0.841
Error	SS=619.3		112	
	MS=5.529			
	G-G Adj DF		59.808	
	H-F Adj DF		71.792	

Time in Open Corridors**First 5 Minutes of Test****Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=40960.000	437.253	1	0.000
	MS=40960.000			
Region	SS=33930.625	4.945	1	0.034
	MS=33930.625			
Drug*Region	SS=39250.225	4.739	1	0.053
	MS=39250.225			
Error	SS=231915.1	4.739	28	
	MS=8282.682			

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=158062.8	19.916	4	0.000
	MS=39515.697			
	G-G Adj DF		2.968	0.000
	H-F Adj DF		3.716	0.000
Week*Drug	SS=3650.563	0.46	4	0.765
	MS=912.641			
	G-G Adj DF		2.968	0.717
	H-F Adj DF		3.716	0.636
Week* Region	SS=21867.438	2.755	4	0.031
	MS=5466.859			
	G-G Adj DF		2.968	0.049
	H-F Adj DF		3.716	0.02
Week*Drug*Region	SS=3112.962	2.755	4	0.814
	MS=778.241			
	G-G Adj DF		2.968	0.762
	H-F Adj DF		3.716	0.693
Error	SS=222215.9		112	
	MS=1984.07			
	G-G Adj DF		83.104	
	H-F Adj DF		104.048	

Second 5 Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=2348.556	0.414	1	0.525
	MS=2348.556			
Region	SS=1446.006	0.255	1	0.618
	MS=1446.006			
Drug*Region	SS=18254.256	3.218	1	0.084
	MS=18254.256			
Error	SS=158838.6		28	
	MS=5672.806			

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=62686.688 MS=15671.672	5.292	4	0.001
Week*Drug	SS=10609.788 MS=2652.447	0.896	4	0.469
Week*Region	SS=7620.338 MS=1905.084	0.643	4	0.633
Week*Drug*Region	SS=4761.338 MS=1190.332	0.402	4	0.807
Error	SS=331689.1 MS=2961.509		112	

Total 10 Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=38316.1 MS=38316.1	1.622	1	0.213
Region	SS=41473.6 MS=41473.6	1.756	1	0.196
Drug*Region	SS=101103.0 MS=101103.0	4.28	1	0.048
Error	SS=661355.6 MS=23619.845		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=418180.9 MS=104545.2	12.768	4	0.000
Week*Drug	SS=16372.65 MS=4093.163	0.5	4	0.736
Week*Region	SS=26684.275 MS=6671.069	0.815	4	0.518
Week*Drug*Region	SS=7151.975 MS=1787.994	0.218	4	0.928
Error	SS=917037.9 MS=8187.838		112	

Corridor Rearing**First 5 Minutes of Test****Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=126.025 MS=126.025	0.291	1	0.594
Region	SS=2325.625 MS=2325.625	5.364	1	0.028
Drug*Region	SS=3080.025 MS=3080.025	7.104	1	0.013
Error	SS=12140.3 MS=433.582		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=6743.162 MS=1685.791	11.37	4	0.000
Week*Drug	SS=73.663 MS=18.416	0.124	4	0.973
Week*Region	SS=590.438 MS=147.609	0.996	4	0.413
Week*Drug*Region	SS=329.288 MS=82.322	0.555	4	0.696
Error	SS=16605.45 MS=148.263		112	

Second 5 Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=22.5 MS=22.5	0.116	1	0.736
Region	SS=792.1 MS=792.1	4.067	1	0.053
Drug*Region	SS=1150.025 MS=1150.025	7.959	1	0.009
Error	SS=5452.75 MS=194.741		28	

Within Group Effects

Effects	Statistic	F	DF	P
Week	SS=3185.9 MS=796.475	7.394	4	0.000
Week*Drug	SS=386.125 MS=96.531	0.896	4	0.469
Week*Region	SS=249.775 MS=62.44	0.58	4	0.678
Week*Drug*Region	SS=182.1 MS=45.525	0.423	4	0.792
Error	SS=12064.5 MS=107.719		112	

Total 10 Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=15.006 MS=15.006	0.015	1	0.903
Region	SS=5417.256 MS=5417.256	5.447	1	0.027
Drug*Region	SS=9287.256 MS=9287.256	9.337	1	0.005
Error	SS=27849.625 MS=994.629		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=19280.537 MS=4820.134	12.014	4	0.000
Week*Drug	SS=769.213 MS=192.303	0.479	4	0.751
Week*Region	SS=896.338 MS=224.084	0.559	4	0.693
Week*Drug*Region	SS=818.213 MS=204.553	0.51	4	0.729
Error	SS=44936.5 MS=401.219		112	

Dark Chamber Stretch Attend Postures**First 5 Minutes of Test****Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=120.756 MS=120.756	0.775	1	0.386
Region	SS=676.506 MS=676.506	4.341	1	0.046
Drug*Region	SS=47.306 MS=47.306	0.304	1	0.586
Error	SS=4363.075 MS=155.824		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=365.163 MS=91.291	5.025	4	0.001
Week*Drug	SS=60.588 MS=15.147	0.834	4	0.506
Week*Region	SS=90.338 MS=22.584	1.243	4	0.297
Week*Drug*Region	SS=34.163 MS=8.541	0.47	4	0.758
Error	SS=2034.55 MS=18.166		112	

Second 5 Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=6.806 MS=6.806	0.125	1	0.726
Region	SS=135.056 MS=135.056	2.484	1	0.126
Drug*Region	SS=0.156 MS=0.156	0.003	2	0.958
Error	SS=1522.075 MS=54.36		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=173.213 MS=43.303	3.74	4	0.007
Week*Drug	SS=36.663 MS=9.166	0.792	4	0.533
Week*Region	SS=16.038 MS=4.009	0.346	4	0.846
Week*Drug*Region	SS=51.688 MS=12.922	1.116	4	0.353
Error	SS=1296.8 MS=11.579		112	

Total 10 Minutes of Test

Effect	Statistic	F	DF	P
Drug	SS=202.5 MS=202.5	0.534	1	0.471
Region	SS=1464.1 MS=1464.1	3.864	1	0.059
Drug*Region	SS=44.1 MS=44.1	0.116	1	0.736
Error	SS=10609.7 MS=378.918		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=864.275 MS=216.069	5.266	4	0.001
Week*Drug	SS=162.625 MS=40.656	0.991	4	0.416
Week*Region	SS=125.9 MS=31.475	0.767	4	0.549
Week*Drug*Region	SS=100.15 MS=25.038	0.61	4	0.656
Error	SS=4595.05 MS=41.027		112	

Illuminated Corridor Stretch Attend Postures**First 5 Minutes of Test****Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=0.025 MS=0.025	0.002	1	0.961
Region	SS=38.025 MS=38.025	3.793	1	0.062
Drug*Region	SS=18.225 MS=18.225	1.818	1	0.188
Error	SS=280.7 MS=10.025		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=59.038 MS=14.759 G-G Adj DF H-F Adj DF	4.876	4 2.6 3.196	0.001 0.008 0.002
Week*Drug	SS=59.913 MS=14.978 G-G Adj DF H-F Adj DF	4.948	4 2.6 3.196	0.001 0.008 0.002
Week*Region	SS=42.163 MS=10.541 G-G Adj DF H-F Adj DF	3.482	4 2.6 3.196	0.01 0.035 0.014
Week*Drug*Region	SS=51.838 MS=12.959 G-G Adj DF H-F Adj DF	4.281	4 2.6 3.196	0.003 0.015 0.005
Error	SS=339.05 MS=3.027 G-G Adj DF H-F Adj Df		112 72.8 89.488	

Second 5 Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=93.025 MS=93.025	2.655	1	0.114
Region	SS=96.1 MS=96.1	2.743	1	0.109
Drug*Region	SS=93.025 MS=93.025	2.655	1	0.114
Error	SS=980.95 MS=35.034		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=68.225 MS=17.056 G-G Adj DF H-F Adj DF	1.909	4 1.088 1.22	0.114 0.564 0.516
Week*Drug	SS=74.35 MS=18.588 G-G Adj DF H-F Adj DF	2.08	4 1.088 1.22	0.088 0.529 0.479
Week*Region	SS=69.025 MS=17.256 G-G Adj DF H-F Adj DF	1.931	4 1.088 1.22	0.11 0.56 0.511
Week*Drug*Region	SS=67.6 MS=16.9 G-G Adj DF H-F Adj DF	1.891	4 1.088 1.22	0.117 0.568 0.52
Error	SS=1000.8 MS=8.936 G-G Adj DF H-F Adj DF		112 30.464 34.16	

Total 10 Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=88.156 MS=88.156	1.286	1	0.266
Region	SS=257.556 MS=257.556	3.743	1	0.063
Drug*Region	SS=187.056 MS=68.819	2.718	1	0.11
Error	SS=1926.925 MS=68.819		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=104.813 MS=26.203 G-G Adj DF H-F Adj DF	2.182	4 1.744 2.048	0.076 0.296 0.227
Week*Drug	SS=234.213 MS=58.553 G-G Adj DF H-F Adj DF	4.875	4 1.744 2.048	0.001 0.048 0.026
Week*Region	SS=122.163 MS=30.541 G-G Adj DF H-F Adj DF	2.543	4 1.744 2.048	0.044 0.232 0.17
Week*Drug*Region	SS=132.413 MS=33.103 G-G Adj DF H-F Adj DF	2.756	4 1.744 2.048	0.031 0.201 0.143
Error	SS=1345.2 MS=12.011 G-G Adj DF H-F Adj DF		112 48.832 57.344	

Corridor Transition Frequency**First 5 Minutes of Test****Between Groups Effects**

Effect	Statistic	F	DF	P
Drug	SS=119.025 MS=119.025	1.04	1	0.317
Region	SS=160.0 MS=160.0	1.398	1	0.247
Drug*Region	SS=384.4 MS=384.4	3.358	1	0.078
Error	SS=3205.15 MS=114.47		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=655.162 MS=163.791	4.599	4	0.002
Week*Drug	SS=220.663 MS=55.166	1.549	4	0.193
Week*Region	SS=237.813 MS=59.453	1.669	4	0.162
Week*Drug*Region	SS=147.912 MS=36.978	1.038	4	0.391
Error	SS=3988.85 MS=35.615		112	

Second 5 Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=22.5 MS=22.5	0.465	1	0.501
Region	SS=0.01 MS=0.01	0.002	1	0.964
Drug*Region	SS=3.025 MS=3.025	0.063	1	0.804
Error	SS=1353.95 MS=48.355		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=446.4	5.167	4	0.001
	MS=111.6			
	G-G Adj DF		3.016	0.002
	H-F Adj DF		3.66	0.000
Week*Drug	SS=93.083	1.077	4	0.369
	MS=23.271			
	G-G Adj DF		3.016	0.359
	H-F Adj DF		3.66	0.272
Week*Region	SS=93.9	0.543	8	0.822
	MS=11.738			
	G-G Adj DF		6.032	0.656
	H-F Adj DF		7.32	0.78
Week*Drug*Region	SS=273.017	1.58	8	0.134
	MS=34.127			
	G-G Adj DF		6.032	0.097
	H-F Adj DF		7.32	0.125
Error	SS=3628.4		168	
	MS=21.598			
	G-G Adj DF		126.672	
	H-F Adj DF		153.72	

Total 10 Minutes of Test**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=29.758	0.118	1	0.734
	MS=29.758			
Region	SS=166.056	0.658	1	0.424
	MS=166.056			
Drug*Region	SS=493.506	1.956	1	0.173
	MS=493.506			
Error	SS=7065.125		28	
	MS=252.326			

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=1860.937 MS=465.234	4.716	4	0.001
Week*Drug	SS=632.838 MS=158.209	1.604	4	0.178
Week*Region	SS=203.038 MS=50.759	0.514	4	0.725
Week*Drug*Region	SS=555.838 MS=138.959	1.408	4	0.236
Error	SS=11049.75 MS=98.658		112	

Initial Corridor Transition Latency**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=65731.556 MS=65731.556	0.478	1	0.495
Region	SS=6039.306 MS=6039.306	1.076	1	0.308
Drug*Region	SS=11611.056 MS=11611.056	2.069	1	0.161
Error	SS=157170.1 MS=5613.217		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=1252.35	0.53	4	0.714
	MS=313.088			
	G-G Adj DF		1.816	0.81
	H-F Adj DF		2.144	0.769
Week*Drug	SS=3269.75	1.383	4	0.244
	MS=817.438			
	G-G Adj DF		1.816	0.48
	H-F Adj DF		2.144	0.405
Week*Region	SS=4039.35	1.709	4	0.153
	MS=1009.838			
	G-G Adj DF		1.816	0.386
	H-F Adj DF		2.144	0.31
Week*Drug*Region	SS=4793.1	2.028	4	0.095
	MS=1198.275			
	G-G Adj DF		1.816	0.31
	H-F Adj DF		2.144	0.238
Error	SS=66189.05		112	
	MS=590.974			
	G-G Adj DF		50.848	
	H-F Adj DF		60.032	

Latency to Dark Chamber Entry**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=119.025	5.484	1	0.027
	MS=119.025			
Region	SS=34.225	1.577	1	0.22
	MS=34.225			
Drug*Region	SS=2.5	0.115	1	0.737
	MS=2.5			
Error	SS=607.75		28	
	MS=21.705			

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=1882.85	34.39	4	0.000
	MS=470.713			
	G-G Adj DF		1.624	0.000
	H-F Adj DF		1.892	0.000
Week*Drug	SS=30.35	0.554	4	0.696
	MS=7.588			
	G-G Adj DF		1.624	0.825
	H-F Adj DF		1.892	0.79
Week*Region	SS=109.15	1.994	4	0.1
	MS=27.287			
	G-G Adj DF		1.624	0.367
	H-F Adj DF		1.892	0.299
Week*Drug*Region	SS=4.25	0.78	4	0.989
	MS=1.063			
	G-G Adj DF		1.624	0.894
	H-F Adj DF		1.892	0.985
Error	SS=1533.0		112	
	MS=13.688			
	G-G Adj DF		45.472	
	H-F Adj DF		52.976	

Dark Chamber Re-Entry Latency**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=372.1 MS=372.1	2.359	1	0.136
Region	SS=230.4 MS=230.4	1.461	1	0.237
Drug*Region	SS=0.000000 MS=0.000000	0.000	1	1.000
Error	SS=4415.8 MS=157.707		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=2643.088	3.961	4	0.005
	MS=660.772			
	G-G Adj DF		1.072	0.258
	H-F Adj DF		1.2	0.212
Week*Drug	SS=595.588	0.893	4	0.471
	MS=148.897			
	G-G Adj DF		1.072	0.812
	H-F Adj DF		1.2	0.784
Week*Region	SS=1159.413	1.737	4	0.147
	MS=289.853			
	G-G Adj DF		1.072	0.607
	H-F Adj DF		1.2	0.562
Week*Drug*Region	SS=258.563	0.387	4	0.817
	MS=64.641			
	G-G Adj DF		1.072	0.936
	H-F Adj DF		1.2	0.926
Error	SS=18684.95		112	
	MS=166.83			
	G-G Adj DF		30.016	
	H-F Adj DF		33.6	

Initial Corridor Re-Entry Latency**Between Group Effects**

Effect	Statistic	F	DF	P
Drug	SS=3330.625 MS=3330.625	0.888	1	0.354
Region	SS=5880.625 MS=5880.625	1.567	1	0.221
Drug*Region	SS=6708.1 MS=6708.1	1.788	1	0.192
Error	SS=105057.4 MS=3752.048		28	

Within Group Effects

Effect	Statistic	F	DF	P
Week	SS=5024.35	2.149	4	0.079
	MS=1256.087			
	G-G Adj DF		1.308	0.433
	H-F Adj DF		1.492	0.373
Week*Drug	SS=3588.0	1.532	4	0.197
	MS=897.0			
	G-G Adj DF		1.308	0.577
	H-F Adj DF		1.492	0.521
Week*Region	SS=4631.0	1.98	4	0.102
	MS=1157.75			
	G-G Adj DF		1.308	0.469
	H-F Adj DF		1.492	0.409
Week*Drug*Region	SS=3223.4	1.378	4	0.246
	MS=805.85			
	G-G Adj Df		1.308	0.619
	H-F Adj DF		1.492	0.566
Error	SS=65477.65		112	
	MS=584.622			
	G-G Adj DF		36.624	
	H-F Adj DF		41.776	