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**Intracerebral Administration of U-69,593 and norBNI in the
Medial Prefrontal Cortex and the Ventral Tegmental Area of
CD-1 Mice: Behavioural Evaluation in the Elevated Plus Maze**

**Thesis Presented to the Faculty of Graduate Studies and Research in Partial
Fulfillment of the Requirements for the Degree of Master of Science
Neuroscience Specialization
Department of Psychology**

Philip M. Wall

Date: Aug. 14, 1997

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Intracerebral Administration of U-69,593 and norBNI in the
Medial Prefrontal Cortex and the Ventral Tegmental Area of CD-1 Mice:
Behavioural Evaluation in the Elevated Plus Maze

submitted by Philip M. Wall B.A. (Hons)

in partial fulfilment of the requirements for
the degree of Master of Science
Neuroscience Specialization
Psychology

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September 10, 1997

Abstract

The putative contributions of the mesocortical dopamine/cholecystokinin system to the control of anxiety-related behaviour in the elevated plus maze was proactively examined via microinfusion of the selective kappa₁ opioid agonist, U-69,593, and non-selective kappa antagonist, norBNI, in the medial prefrontal cortex and ventral tegmental area of CD-1 mice. U-69,593 produced an anxiolytic behavioural profile from the medial prefrontal cortex, effects for the most part not evident from the ventral tegmental area. NorBNI produced a dose-dependent anxiogenic behavioural profile from the medial prefrontal cortex, effects not evident from the ventral tegmental area. Results are discussed relative to a) the possible influence kappa opioids may exert on neurochemical responsivity to anxiety-provoking environments in the medial prefrontal cortex and lack thereof in the Ventral tegmental area, b) possible mesocortical influence on anxiety-related behavioural responsivity and c) the utility of the behavioural indices typically analyzed in the elevated plus maze .

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Introduction

Anxiety-related disorders have become increasingly prevalent in our society in recent times, with research data showing that approximately 15% of the general population will experience at least one lifetime pathological anxiety episode (Regier et al. 1988; Eaton et al. 1991). In addition, many behavioural, psychological and/or physiological indices of anxiety are apparent in numerous psychological disorders (Edwards, 1991; Leon et al. 1995). For example, it is difficult to distinguish between melancholic depression and generalized anxiety disorder, since the etiology, symptom constellations and responsiveness to treatment often overlap (Clayton et al. 1991; Edwards, 1991; Coryell et al. 1992; Frances et al. 1992; Kendler et al. 1992; Kendler, 1996; Andrews, 1996; Brown et al. 1996; Sartorius et al. 1996; Goldberg, 1996). Accordingly, some investigators have described melancholic depression as an organized state of anxiety attached principally to the self, coupled with lowered self esteem, a profound sense of worthlessness and hopelessness, and motivational deficits (Johnson et al. 1992). Generalized anxiety disorder is typically characterized by extreme anxiety and nonspecific worry about present and future life events (Yonkers et al. 1996). These symptom constellations are frequently comorbid with increased sympathetic arousal (e.g., increased heart rate, blood pressure and respiration), hypervigilance, as well as inhibition of vegetative functions (e.g., decreased gastric motility) during stressful conditions. Such observations have prompted some investigators to posit that reduced ability to cope with stressors is common among individuals compromised with anxiety/depressive disorders (Johnson et al. 1992; Andrews, 1996). Accordingly, it has been suggested that abnormalities in the counterregulation of the stress response in the hypothalamus-pituitary-adrenal axis (HPA) resulting in corticotropin-releasing hormone (CRF) and glucocorticoid

(GC), or central catecholamine (CA) hypersecretion, could be two common pathophysiological underpinnings (Smith et al. 1989; Sapolsky, 1996). In effect, it is conceivable that arousal emerges as dysphoric hyperarousal, vigilance is replaced by hypervigilance and insomnia, and assertiveness emerges as excessive cautiousness (Chrousos and Gold, 1992).

Traumatic life events in childhood (Roth, 1996; Brown et al. 1996), acute stressful events and/or chronic psychosocial adverse conditions (Post, 1992; Yonkers et al. 1996; Brown et al. 1996), as well as psychoactive drug abuse (Edwards, 1991) have been associated with anxiety and/or depression in humans. There has consequently been considerable research associated with investigations of the effects of various acute and/or chronic environmental events which may contribute to the expression of some of the symptoms of anxiety and depression. These data suggest that stressful environmental factors may not only trigger both types of psychopathology (Johnson et al. 1992; Frances et al. 1992; Yonkers et al. 1996), but may also sensitize neurochemical change favouring such psychopathology (Post, 1992). Genetic predispositional influences on the induction of psychopathology have also been investigated. These data suggest that children born to at least one parent with anxiety and/or depression may have increased risk of expressing one or both of these disorders (Weissman, 1990; Kendler et al. 1992), particularly when faced with stressful life events (Brown et al. 1996). Moreover, some investigators suggest that depression- and anxiety-related disorders may stem from a common genetic underpinning (Kendler et al. 1992; Kendler, 1996).

Infrahuman subjects also respond to stressful conditions or aversive environmental events with anxiety-related behavioural and autonomic symptoms reminiscent of those

observed in humans. In addition, rats and mice which display more anxious behaviour in various paradigms, compared to non-anxious control animals, appear neurochemically and/or behaviourally vulnerable to natural or experimentally-induced environmental stressors (Robertson et al. 1978; Robertson, 1979; Harro et al. 1990a; Vasar et al. 1993a, 1994). Numerous animal paradigms have been developed to assess the anxiogenic effects of environmental stimuli in rats and mice, to evaluate the influence of anxiogenic or anxiolytic agents and to elucidate the neural mechanisms underlying the induction and expression of psychopathology (Handley and McBlane, 1993; Hamon, 1994; Stout and Weiss, 1994; File, 1996). For example, some animal models of anxiety combine conditioning and aversive stimulation in appetitively-motivated conflict paradigms (Shephard, 1986 for review; Dawson and Tricklebank, 1995), while other laboratories have assessed performance in novelty/exploration paradigms (Rodgers and Cole, 1993a; Ladurelle et al. 1995). Novelty/exploration models such as the elevated plus maze capitalize on the fear rats and mice exhibit for unprotected open spaces (Handley and Mithiani, 1984a; Pellow and File, 1986; Lister, 1987; Handley et al. 1993; Treit et al. 1993; Dawson and Tricklebank, 1995), while the light/dark test evaluates fear of protected highly illuminated arenas (Crawley, 1981; Costall et al. 1989; Cancela et al. 1995). Still other models assess anxiety by varying the nature of fear-inducing stimuli. For example, the social interaction test employs novelty and illumination to assess the tendency of rats to avoid contact with recently introduced rats in a novel environment (File and Hyde, 1979; File, Pellow and Braestrup, 1985). Likewise, the open field test evaluates the latency of rats and mice to venture away from the protective edges of a novel open arena (thigmotaxis) (Simon et al. 1994), whereas the defensive/withdrawal test evaluates the latency of rats and mice to venture out of a protective

chamber positioned in the middle of an open arena (Yang et al, 1990). Taken together, such paradigms are useful animal models of anxiety since such tasks appear to induce fear (anticipatory anxiety) as an analogy to human generalized anxiety disorder (Green, 1991).

Several laboratories impose stressor manipulations to provoke anxiogenic responses in animals or to augment anxiogenic behaviour provoked by novelty or conflict situations. These manipulations may include restraint, footshock, tailpinch, tailshock, forced swim, isolation and social defeat among others. For example, laboratory animals often respond to restraint stress with tonic immobility (stress-induced immobility) (Moriarty, 1995) or acute footshock by emitting ultrasonic vocalizations (Jolas et al, 1995). Rat pups separated from the dam also emit ultrasonic vocalizations (Insel and Winslow, 1991). In effect, paralleling the anxiety provoked by exposure to novelty or conflict situations, stressors, or cues associated with stressors, produce anxiety-like behavioural responses in animals. Conditioned responses to stress (conditioned emotional response) or stress-induced immobility have been employed as animal models of either anticipatory anxiety (Nabeshima et al, 1992; Yamada and Nabeshima, 1995; Yoshioka et al, 1995) or depression (Katoh et al, 1990, 1991, 1992; Lucki, Singh and Kreiss, 1994; Tanda et al, 1995). For example, rats exhibit freezing behaviour following placement into a chamber previously associated with footshock (Yoshioka et al, 1995) or the fear potentiated startle paradigm assesses the amplitude of a reflex in the presence of a cue previously paired with footshock (Davis, 1992; Davis et al, 1993). Stressors can also augment anxiety-like behaviour in the elevated plus maze or the light/dark task. For example, the stress of social defeat in male mice or the exposure of rats or mice to the scent of a predator or an aggressive conspecific not only reduces open arm entries and time dependent measures, but also enhances protected risk

assessment behaviours such as closed arm returns, stretch attend postures, and head dipping (Zangrossi Jr. and File, 1992; Rodgers and Cole, 1993a). Conversely, acute cold water immersion, acute restraint, open or closed arm confinement and acute inescapable footshock fail to affect open arm preference in the elevated plus maze in rats (Handley and McBlane, 1993). In contrast to the failure of various acute stressors to augment anxiety in the elevated plus maze (Handley and McBlane, 1993), acute restraint or brief uncontrollable footshock enhances anxiety in rats in the light/dark paradigm (Cancela et al, 1995). Taken together, different subtypes of anxiety (or symptom clusters) may be induced by specific animal models, which may be differentially affected by stressors (File, 1996). In addition, different stressors, novelty/conflict exposure, or various combinations of such aversive events provoke concomitant neurochemical responses in specific sites within the brain and certain brainstem nuclei.

The Neuropharmacology and Neurobiology of Anxiety-Like Behavioural Responses to Stressful Events and Anxiogenic Drug Administration

Numerous neuroanatomical, electrophysiological, neurochemical and in vivo microdialysis data have revealed that several neurochemical pathways may simultaneously respond to specific stressor regimes, anxiogenic drug administration, as well as novelty/conflict exposure. For example, brainstem nuclei associated with anxiety include the nucleus of the solitary tract (Terreberry and Neafsey, 1983; Vasar et al, 1993a, 1993b, 1994; Bradwejn and Koszicki, 1994), the dorsolateral tegmental nucleus (Byrum and Guyenet, 1987), the rostral ventrolateral medulla (Wallace et al, 1989; Van der Plas et al, 1995; Thomas and Spyer 1996), the parabrachial nucleus (Ottersen, 1981; Ross et al, 1981; Jhamandas et al, 1996) and the dorsal motor nucleus of the vagus (Wallace et al, 1989; Ter

Horst et al. 1991). These brainstem nuclei regulate heart rate, blood pressure, pulmonary function and gastric motility (Jordan and Spyer, 1986; Chrousos and Gold, 1992; Johnson et al. 1992; Pezzone et al. 1993). In addition, visceral interoceptive and environmental exteroceptive stimuli which enhance electrophysiological activity in brainstem nuclei including the nucleus of the solitary tract and the rostral ventrolateral medulla (van der Kooy et al. 1984; Wallace et al. 1989), also enhance the electrophysiological activity in the ventral tegmental area (VTA) (Mantz et al. 1988a, 1988b; Biggio et al. 1990; Kaneyuki et al. 1991; Goldstein et al. 1996), the locus coeruleus (Ter Horst et al. 1991; Nutt and Lawson, 1992), the dorsal periaqueductal grey (Neafsey et al. 1986; Guimarães et al. 1991; Audi et al. 1991; Van der Plas et al. 1995), the dorsal and median raphe nuclei (File, Gonzalez and Andrews, 1996; Graeff et al. 1996) and limbic forebrain structures including the posterior nucleus accumbens (Zaborski et al. 1985; Wang et al. 1992; Derrien et al. 1994; Ladurelle et al. 1995), the central nucleus of the amygdala (Ottersen, 1981; Zardetto-Smith and Gray, 1995; Jhamandas et al. 1996), the paraventricular nucleus of the hypothalamus (PVN) (Pittman and Franklin, 1985; Pacak et al. 1992) and the medial prefrontal cortex (Sesack and Bunney, 1989; Takagishi and Chiba, 1991; Kaneyuki et al. 1991; Goldstein, Rasmussen, Bunney and Roth, 1996).

Various neurochemicals colocalized within these central sites are responsive to stressful stimuli and which appear to be involved in the induction, expression or reduction of anxiety-related behavioural symptoms, include gamma aminobutyric acid (GABA) (File, 1993), serotonin (5-HT) (Taylor and Moon, 1991; Handley and McBlane, 1993; Inoue et al. 1993; Yamada and Nabeshima, 1995; Griebel, 1995; Handley, 1995 for review; Stanford, 1996), norepinephrine (NE) (Charney et al. 1989; Nutt and Lawson, 1992; Roth, 1996),

epinephrine (E) (Wallace et al. 1989; Zardetto-Smith and Gray, 1995), dopamine (DA) (Taylor, 1982; Kaneyuki et al. 1991; File, 1996; Goldstein et al. 1996; Rodgers et al. 1996), corticotropin-releasing factor (CRF) (Johnson et al. 1992; Pezzone et al. 1993), cholecystokinin (CCK) (Hughes et al. 1990; Vasar et al. 1993a, 1993b, 1994) and opioid peptides such as dynorphin (Nabeshima et al. 1992; Olson et al. 1993, 1995; Privette and Terrian, 1995), and the enkephalins (Yamada and Nabeshima, 1995), among others. These neurochemicals are released in the brainstem, hindbrain, midbrain, forebrain limbic areas and the prefrontal cortex (Deutch and Roth, 1990; Inoue et al. 1993) following encounters with stressful environmental events and/or anxiogenic interoceptive stimuli. In effect, multiple neurochemical responses to stressful environmental events appear to not only involve activation of the sympathetic nervous system from the brainstem, but also activation of the neuroendocrine axis from the hypothalamus or the brainstem (Harbuz et al. 1991; Pezzone et al. 1993). Sympathetic nervous system activation involves the catecholamines (CAs) NE and E colocalized in the brainstem (Jordan and Spyer, 1986; Thomas and Spyer, 1996), colocalized CRF in the PVN and the central nucleus of the amygdala (Heinrichs et al. 1992) and colocalized CCK in the nucleus of the solitary tract, the basolateral and central amygdala, the medial prefrontal cortex and the ventral tegmental area (Hökfelt et al. 1985). In addition, neuroendocrine activation involves CRF (Imaki and Vale, 1993), 5-HT from the dorsal raphe (Laflamme et al. 1996), CAs from the brainstem (Harbuz et al. 1991) and CCK from the cortex or brainstem nuclei (Ladurelle et al. 1995). Taken together, although various neurochemicals appear to participate in anxiety-related behaviours within defined neurochemical pathways, with the exception of GABA_A, some 5-HT and CCK receptor systems, the neurobiology and behavioural pharmacology of anxiolysis remains to be

defined. Along with stressor-induced activation of the sympathetic nervous system and neuroendocrine axes, it would appear that the severity of stressful events and/or aversive situations can differentiate both neurochemical and behavioural responsivity within different animal models (Deutch and Cameron, 1992; Yasham and Stewart, 1994). Neurochemically- and behaviourally-differentiated responses in various animal models of anxiety are also evident following anxiogenic drug administration. Peripheral or central anxiogenic drug administration can enhance anxious behaviours in various animal models of anxiety (Handley and McBlane, 1993), reminiscent of the anxiogenic effects of some stressor pretreatments in such paradigms. The apparent similarities between drug-induced and stressor-induced anxiety in various animal models have raised the possibility that the neurochemical concomitants of anxiety from such sources may functionally overlap. Several questions are raised however: 1) Do anxiogenic drugs distinguish subtypes of anxiety in different species in a similar fashion to specific stressor regimes? 2) Do central sites contribute differentially to the expression of anxiety in specific anxiety paradigms? 3) Can site-specific central administration of various putative anxiolytic drugs similarly differentiate anxiety subtypes and accordingly reduce behavioural symptoms in only certain animal models, or in anxiety paradigms in general?

Central GABA/Benzodiazepine Activity and Anxiety-Related Behaviour

Systemic administration of benzodiazepines (BDZs), GABA_A receptor agonists, are anxiolytic in most animal models of anxiety (Green, 1991). Typically, animal models of anxiety have been validated by the demonstration of anxiolytic effects following BDZ administration, thus have provided a standard against which putative anxiolytics have been assessed (Shephard, 1986; Treit and Pesold, 1990; Green, 1991). This anxiolytic standard

has been challenged however, owing to accumulating data which suggest that BDZs do not invariably antagonize stressor-induced increases in corticosterone in mice (Groenink et al. 1996), nor do BDZs attenuate CRF-induced anxiety-like behaviour in rats (Yang et al. 1990). In addition, BDZs appear to address only some aspects of anxiety-related behaviour in rats in the elevated plus maze (File, 1993). Nevertheless, stressors and novelty/conflict exposure exert profound effects on GABA release and GABA_A receptor binding in rats (Rago et al. 1991; File, 1996). For example, File (1993) has shown that elevated plus maze exposure increases GABA release and decreases GABA_A receptor binding in rat brain. Moreover, anxious rats (assessed in the elevated plus maze) have fewer GABA_A binding sites in the cortex, than non-anxious control animals (Harro et al, 1990a; Rago et al. 1991). In like fashion, Robertson et al (1978) found that highly anxious Maudsley reactive rats had lower BDZ binding sites in the hippocampus, hypothalamus, midbrain, medulla/pons and spinal cord. These data suggest that animals which exhibit elevated levels of anxiety show reduced GABA_A receptor binding in some central sites. In contrast to the anxiety-like behavioural and neurochemical effects of elevated plus maze exposure, isolation rearing produced anxiogenic effects in the light/dark test, but did not effect GABA_A receptor binding in the frontal cortex, hippocampus or the amygdala in rats (Morinan et al, 1992). These data suggest that stressful manipulations exert anxiogenic effects in some paradigms independent of GABA_A receptor alterations. However, systemic administration of the BDZ inverse agonist, FG-7142, augments anxiety in the elevated plus maze in rats (Pellow and File, 1986; Lister, 1987; File, 1993), the light/dark paradigm in rats (Cancella et al, 1995), the social interaction and hole board exploration tasks in rats (File et al, 1985), as well as enhances shock induced suppression of drinking in rats (Corda et al, 1983; Hill et al, 1992). Moreover,

β -carboline interact with DA, NE, CCK and 5-HT in the production of anxiety-like behaviour. For example, systemic FG-7142 elevates 3,4-dihydroxyphenylacetic acid (DOPAC) levels and the DOPAC/DA ratio in the prefrontal cortex in rats (Tam and Roth, 1990), as well as increases DA release in the medial prefrontal cortex in rats (Gresch et al, 1994). Systemic FG-7142 also increases NE metabolism in the hypothalamus, amygdala, thalamus and cortex in rats (Ida et al, 1991). In addition, FG-7142 augments CCK_B receptor binding in the frontal cortex in rats (Harro et al, 1990b, 1993). It would appear that these concomitant neurochemical interactions, produced by systemic FG-7142, occur during anxiety-like behaviours in the elevated plus maze, the light\dark task or conflict situations, intriguingly reminiscent of the behavioural symptoms augmented by some stressor manipulations in such paradigms. It is therefore noteworthy that β -carboline can mimic, at least in part, stressor-induced anxiety-related behavioural and neurochemical responsivity in infrahuman subjects.

In contrast to the behavioural effects of BDZ inverse agonists in infrahuman anxiety paradigms, systemic GABA_A receptor agonists antagonize anxiogenic behavioural responsivity, as well as the anxiogenic effects of stressors or anxiogenic drug administration in conditioning, novelty or conflict paradigms (Cancella et al, 1995). For example, BDZs augment punished responding for food reinforcement (Shephard, 1986; Handley and McBlane, 1993), as well as in novelty-induced suppression of feeding in rats (Bodnoff et al, 1989). Benzodiazepines also decrease anxiogenic behaviour in rats and mice provoked by a single exposure to the elevated plus maze (Rodgers et al, 1992; File, 1993; Agmo et al, 1995; Dawson and Tricklebank, 1995; Rogers et al, 1995), reduce conditioned freezing in rats (Yoshioka et al, 1995), as well as increase exploration in the light-dark task in rats

(Costal et al. 1989). In addition, systemic BDZs interact with DA, CRF, CCK and 5-HT under basal and stressful conditions. For example, systemic BDZs increase DA release in the mesocortical system under basal conditions in rats (Tam and Roth, 1990), but block stressor- or β -carboline-induced increased DOPAC/DA ratios in the mesocortex (Lavielle et al. 1978; Tam & Roth, 1990), as well as attenuate the anxiogenic effects of systemic CCK in rats (Harro et al. 1993). Systemic BDZ administration also decreases footshock-induced CRF mRNA accumulation in the PVN in rats, and attenuates the stressor-induced HPA axis activation in rats (Imaki and Vale, 1993). Furthermore, the behavioural indices of anxiety in rats, produced by acute withdrawal from chronic BDZ administration, not only is blocked by systemic administration of the CCK_B receptor antagonist, CI-988 (Singh et al. 1992), but also increases CCK_B receptor density in the frontal cortex, effects reminiscent of FG-7142 treatment (Harro et al. 1990b, 1993). Interestingly, the BDZ antagonist, flumazenil, antagonized the anxiogenic effects of the systemically-administered CCK_B agonist, CCK-8US, in the elevated plus maze in rats (Chopin and Briley, 1993), but was ineffective in blocking the intravenously-administered CCK_B agonist CCK-4-induced panic symptoms in healthy human volunteers (Bradwejn et al. 1994). These data suggest that GABA_A receptor antagonists may reduce only some behavioural symptoms of anxiety, and perhaps only in some species.

There is some evidence that GABA_B receptor mechanisms may exert neurochemical effects in brain areas involved in anxiety-like behavioural responding. For example, central administration of the GABA_B agonist, baclofen, but not the GABA_A agonist, muscimol, reduced DA release following microinjection in the medial prefrontal cortex in rats (Santiago et al. 1993). Interestingly, the GABA_B antagonist, phaclofen, increased DA release in this

cortical site. Moreover, baclofen inhibits the release of CCK from cortical synaptosomes in rats (Gemignani et al. 1994) and neocortical synaptosomes in humans (Raiteri et al 1996). In this respect, it is noteworthy that baclofen has been reported to exert anxiolytic activity in both animals and humans (Breslow et al. 1989; File et al. 1992; Andrews and File. 1993). Specifically, Andrews and File (1993) demonstrated that baclofen was anxiolytic in the elevated plus maze in handling-naïve rats. These data are noteworthy in view of the observation that handling-naïve rats exhibit significantly increased cortical 5-HT activity under stressful conditions (File et al. 1990) and baclofen decreases 5-HT release from the frontal cortex in mice (Gray and Green. 1987).

In addition to the central effects exerted by GABA_B receptor activation on DA, CCK and 5-HT activity, central BDZ administration in the dorsal raphe nucleus decreases 5-HT cell firing in rats during conditioned cue exposure (Yoshioka et al. 1995). Similarly, intravenously-administered BDZs suppress dorsal raphe 5-HT cell firing in rats at anxiolytic doses (Laurent et al. 1983). Finally, iontophoretic application of GABA in the dorsal raphe, amygdala and hippocampus inhibits 5-HT neuronal activity, an effect which is enhanced by systemic BDZs (Gallager. 1978). These data demonstrate that GABA_A and GABA_B receptor activity in brain sites associated with the induction of anxiety-related behaviour can differentially attenuate anxiety-like behavioural and neurochemical responses to stressful environmental events in some anxiety paradigms in infrahuman subjects.

Central Serotonin Activity and Anxiety-Related Behaviour

It has been well documented that stressful environmental events alter central 5-HT activity (Takada et al. 1996). For example, conditioned fear increases extracellular 5-HT concentrations and metabolism in the medial prefrontal cortex in rats during freezing

behaviour (Yoshioka et al. 1995). More recently, Takada et al (1996) demonstrated that foot shock did not alter central 5-HT levels in the rat, but did increase 5-hydroxyindole acetic acid (5-HIAA) concentrations in the hypothalamus, midbrain and cortex. Moreover, isolation rearing has been shown to sensitize potassium (K^+)-evoked 5-HT release in the prefrontal cortex in rats (Crespi et al. 1992). In addition, Wright et al (1992) have shown that elevated plus maze exposure increases 5-HT release in the ventral hippocampus in rats. Interestingly, the dorsal raphe in rats is activated by projections from the central nucleus of the amygdala (Davis. 1992), as well as from the nucleus of the solitary tract (Peuranen et al. 1995).

In a similar fashion to the anxiogenic effects induced by GABA_A receptor perturbations, agents which enhance 5-HT transmission including 5-HT₂ and 5-HT_{1C} agonists, or acutely administered 5-HT reuptake inhibitors, such as fluoxetine, exert anxiogenic effects in the elevated plus maze in rats (Handley and McBlane, 1993; Yoshioka et al. 1995). Conversely, pharmacological manipulations that decrease 5-HT transmission typically exert an anxiolytic influence (Vasar et al. 1993a; Hamon, 1994). For example, the 5-HT_{1A} receptor partial agonists, 8-OH-DPAT and ipsapirone, reduce ultrasonic vocalizations in rats following footshock (Jolas et al. 1995); whereas the 5-HT_{1A} partial agonist, buspirone, reduces immobility in the forced swim test in rats (Lucki et al. 1994) and increases exploratory behaviour in the elevated plus maze in rats (Dawson and Tricklebank, 1995). Furthermore, buspirone appears to be as effective as BDZs in the treatment of generalized anxiety disorder in humans (Robinson, 1991). It is noteworthy that buspirone is also a D₂ receptor agonist (Weissman, 1991) and α_2 adrenergic antagonist (Lucki, Singh and Kreiss, 1994). More recently, the 5-HT_{1A} agonist, MKC-242, was anxiolytic in the punished suppression of drinking paradigm in rats (Abe et al. 1996). The 5-HT₂ antagonists, ritanserin

and ketanserin, were also anxiolytic in the elevated plus maze in rats (Critchley and Handley, 1987). The 5-HT₃ receptor antagonist, tropisetron, reduces conditioned freezing in rats (Yoshioka et al. 1995) and is anxiolytic in the social interaction and light-dark tasks in rats (Costall and Naylor, 1991). Interestingly, 5-HT₃ antagonists appear to possess rather limited anxiolytic effects in the elevated plus maze in rats (Andrews and File, 1993), or have no effect in the elevated plus maze in mice (Rodgers et al. 1995). These data suggest that different animal models of anxiety may prompt the expression of diverse anxiety profiles that can be distinguished by the putative anxiolytic agent employed (File, 1996).

In addition to GABA/5-HT interactions in response to stressful environmental events, 5-HT may interface with DA, CRF and CCK in response to stressor imposition or novelty/conflict exposure. For example, the 5-HT₃ antagonist, ICS 205-930, but not diazepam, prevented DA release in the nucleus accumbens and prefrontal cortex induced by restraint stress in freely moving rats (Imperato et al, 1990). More recently, the 5-HT agonist, dex-fenfluramine, activated CRF mRNA in the PVN in rats (Laflamme et al. 1996). Interestingly, CRF neurons in the PVN receive 5-HT input from the dorsal raphe nucleus in rats (Halliday et al. 1995). Evidence is also accumulating to support an interaction between 5-HT and CCK in the production of anxiety (Vasar et al. 1993a). In this respect, 5-HT has been shown to facilitate CCK release in the cortex and nucleus accumbens in rats (Paudice and Raiteri, 1991), an effect mediated by presynaptic 5-HT₃ receptor activation on CCK release sites. The 5-HT₃ receptor antagonist, ondansetron, antagonized the anxiogenic effects of the CCK agonist, caerulein, in the elevated plus maze in rats (Vasar et al. 1993a). The anxiolysis produced by ondansetron following systemic caerulein administration contrasts with the absence of an anxiolytic effect of 5-HT₃ antagonists in the elevated plus maze

among rats (Andrews and File, 1993) and mice (Rogers et al, 1995) when administered alone. In addition to the detection of central 5-HT₃ receptors on CCK release sites in the nucleus accumbens and prefrontal cortex in rats, 5-HT₃ receptor density is also conspicuous in the nucleus of the solitary tract (Pratt et al, 1990), prompting some investigators to suggest that the nucleus of the solitary tract may also contribute to the behavioural effects associated with systemic caerulein and ondansetron administration in the elevated plus maze (Vasar et al, 1993a). Costal et al (1990) have also demonstrated anxiolytic effects in rats following microinfusion of 5-HT₃ antagonists in the central nucleus of the amygdala, as well as in the dorsal raphe nucleus. It would appear that stressor- or novelty/conflict-induced 5-HT transmission may exert anxiogenic effects in some central sites, at least in part, by promoting CCK release. In this respect, it is conceivable that stressor induced interactions between 5-HT and CCK in the medial prefrontal cortex and the nucleus accumbens in rats (Paudice & Raiteri, 1991; Raiteri, Paudice and Vallebuona, 1993), the nucleus of the solitary tract in rats (Vasar et al, 1993a), the dorsal periaqueductal grey (Audi et al, 1991), as well as the dorsal raphe nucleus and the central nucleus of the amygdala in rats (Costal et al, 1990), may site-specifically differentiate anxiety subtypes and/or symptoms in different animal models and/or specific stressor regimes.

Central Cholecystokinin Activity and Anxiety-Related Behaviour

The activation of CCK_B receptor mechanisms, in response to stressful environmental events, has been implicated in the production of anxiety in animals (Harro and Vasar, 1991; Hughes et al, 1991; Rex and Marsden, 1994; Beinfeld, 1995) and in human subjects (Bradwejn and Koszycki, 1994). For example, systemic administration of the CCK_B agonist, CCK-4, induced panic attacks in panic patients (Bradwejn et al, 1990), as well as in healthy

volunteers (de Montigny, 1989). In a like manner, stressors such as footshock increase CCK release in the nucleus accumbens, amygdala and the mesocortex in rats (Harro et al, 1993). More specifically, the activation of CCK_B receptors in the medial prefrontal cortex in rats following footshock or elevated plus maze exposure may be associated with anticipatory anxiety (Harro et al, 1990a). Furthermore, Harro et al (1996) demonstrated that the exposition of rats to other rats undergoing decapitation increased CCK_B receptor binding in the frontal cortex. Interestingly, Vasar et al (1993b) have shown that socially-isolated rats exhibit significantly reduced exploratory activity in the elevated plus maze compared to group-housed animals. Moreover, such anxiety-related behavioural responsivity was correlated with increased CCK_B receptor density in the prefrontal cortex, but not in mesolimbic nuclei or the hippocampus (Vasar et al, 1993b). In fact, paralleling central BDZ binding site differences detected among anxious and non-anxious rats and mice, anxious rats evaluated in the elevated plus maze exhibit a higher density of CCK_B receptors in the prefrontal cortex relative to non-anxious counterparts (Harro et al, 1990a).

Systemic CCK-4 was anxiogenic in the elevated plus maze in rats (Harro and Vasar, 1991; Rex et al, 1994) and guinea pigs (Rex and Marsden, 1994). Furthermore, Rex et al (1994) demonstrated that systemic CCK-4 increased ultrasonic vocalizations in rat pups separated from the dam, reduced feeding in a novelty suppressed feeding conflict test and augmented anxiety induced by the light/dark task in rats. Systemically-administered caerulein was also anxiogenic in the elevated plus maze in mice (Harro et al, 1990c). In contrast, systemic administration of the CCK_B antagonist, CI-988, was anxiolytic in an appetitively-motivated conflict test in squirrel monkeys (Powell and Barrett, 1991). Systemic CI-988, as well as another CCK_B antagonist, PD 135158, were also anxiolytic in the

light/dark task in mice, the social interaction test and elevated plus maze in rats, as well as in the marmoset human threat test (Costall et al. 1991; Derrien et al. 1994a; Belzung et al. 1994). In a like manner, systemic treatment with the CCK_B antagonist, L-365,260, was anxiolytic in the elevated plus maze in mice (Rataud et al. 1991), as well as in the elevated plus maze and conditioned suppression of drinking test in rats (Ravard and Dourish, 1990). In addition, the anxiogenic behavioural effects produced by systemic caerulein (Männistö et al. 1994) or systemic CCK-4 (Haro and Vasar, 1991) in the elevated plus maze in rats were antagonized following systemic L-365,260 pretreatment. Systemic L-365,260 also dose-dependently attenuates fear potentiated startle in rats (Josselyn et al. 1995).

In addition to the anxiety-provoking properties of systemic CCK_B receptor agonist administration, intracranial anxiogenic effects have been demonstrated in several animal models of anxiety. For example, intraventricularly- or intracerebrally-administered CCK-8S, CCK-8US or CCK-4, in the nucleus accumbens or the central nucleus of the amygdala increased fear motivation and/or arousal in an active/passive avoidance task in rats (Fekete et al. 1984). In a like manner, Singh et al (1991) demonstrated that intraventricular administration of the CCK_B agonist, pentagastrin, or caerulein, was anxiogenic in the elevated plus maze in rats, the light/dark task in mice, as well as in an appetitively-motivated conflict test in rats. The anxiogenic effects of intraventricular pentagastrin or caerulein administration in these particular anxiety paradigms were also blocked by systemic CI-988 (Singh et al. 1991). Daugé et al (1989) have demonstrated that CCK-8S, microinjected in the posterior nucleus accumbens, was anxiogenic in the elevated plus maze in rats, effects blocked by the systemically-administered CCK_A antagonist, devazepide. Derrien et al (1993) also demonstrated that CCK-8S, microinjected in the posteromedian nucleus accumbens, was

anxiogenic in the elevated plus maze and the 4-hole box exploration task in rats. These behavioural alterations were suppressed by 6-hydroxydopamine (6-OHDA) lesions of the accumbens, and also blocked by the D₂ receptor antagonist, sulpiride, microinjected into the same site. More recently, *in vivo* microdialysis has revealed that microinjected CCK-8S-induced DA efflux in the posterior nucleus accumbens was correlated with an enhanced anxiety-like behavioural profile in the elevated plus maze among rats pre-exposed to a hole board exploration task (Ladurelle et al, 1995).

Bilateral microinjection of CCK-8S in the central nucleus of the amygdala also augments anxiety-like behaviour in the elevated plus maze in rats (Belcheva et al, 1994). Similar findings from other laboratories prompted some investigators to suggest that the amygdala may be an anxiogenic site for CCK_B agonists (e.g., see Hamilton and Freeman, 1995). Moreover, electrical stimulation of the central amygdaloid nucleus in rats increases heart rate, blood pressure, respiratory rate, plasma CA and corticosterone concentrations, as well as enhances acoustic startle (Wallace et al, 1989; Davis, 1992). The central nucleus of the amygdala (van der Kooy et al, 1984; Wallace et al, 1989), as well as adrenergic neurons from the rostral ventrolateral medulla (Ross et al, 1981), innervate the nucleus of the solitary tract. And perhaps not surprisingly, electrical stimulation of (or intracerebral drug administration in) the nucleus of the solitary tract (Jordan and Spyer, 1986) or the ventrolateral medulla (Dampney et al, 1982) increases blood pressure and heart rate in rats and other animals (Jones and Brody, 1992; Van der Plas et al, 1995; Thomas and Spyer, 1996). In addition, iontophoretic application of CCK-4 in the nucleus of the solitary tract increases neuronal firing and respiratory frequency in cats (Denavit-Saubié et al, 1985). Moreover, Branchereau et al (1992) observed that CCK_B agonists enhanced

electrophysiological activity in the nucleus of the solitary tract in rats. It is conceivable, for example, that CCK_B receptor activation on CA cells in the nucleus of the solitary tract (Bradwejn and Koszycki, 1994) or the rostral ventrolateral medulla can account for some of the symptoms of anxiety (Nicholas, Hökfelt and Pieribone, 1996).

In addition to the stressor-induced neurochemical interactions involving GABA, 5-HT, E and NE, CCK has also been shown to interact with DA in response to stressors or novelty/conflict exposure (Crawley, 1991; Derrien et al, 1993; Ladurelle et al, 1995). Cholecystokinin colocalization has been detected in a subset of VTA DA neurons which project to the posterior nucleus accumbens, central nucleus of the amygdala and the medial prefrontal cortex (Hökfelt et al, 1980a, 1980b, 1985). Interestingly, the anterior nucleus accumbens receives CCK innervation from the medial prefrontal cortex and the basolateral amygdala (Zaborski et al, 1985); whereas the posterior nucleus accumbens receives CCK innervation from the nucleus of the solitary tract (Wang et al, 1992), in addition to the mixed CCK/DA projections from the VTA and substantia nigra pars compacta (Hökfelt et al, 1985). Microinjected CCK-8S in the posteromedian nucleus accumbens increases DA release concomitant with anxiogenic responses in the elevated plus maze in rats (Ladurelle et al, 1995). It remains to be verified whether such behavioural responsivity in the elevated plus maze follows indirectly from CCK-induced DA release in the posteromedian nucleus accumbens, the direct influence of CCK-8S on CCK_A receptors in this accumbal region, or is not at all connected to DA/CCK interactions in the nucleus accumbens. Conceivably, since CCK is colocalized with DA in a subset of neurons in the VTA, stressor- and/or novelty/conflict-induced activation of such DA/CCK neurons may simultaneously enhance CCK and DA release in the posteromedian nucleus accumbens, central nucleus of the

amygdala and the medial prefrontal cortex. It is noteworthy that the systemically-administered non-selective CCK agonist, ceruletide, attenuated DA turnover in the prefrontal cortex and nucleus accumbens in rats, but only after the mesocortical and mesolimbic DA pathways were neurochemically enhanced (Kuroki et al, 1992). These data suggest opposing roles for CCK_B and CCK_A receptors on DA activity in two brain areas suggested to be involved in anxiety-related behaviour (e.g., CCK_B receptor activation reduces DA activity in the prefrontal cortex while CCK_A receptor activation increases DA activity in the posteromedian nucleus accumbens) (Kuroki et al, 1992; Ladurelle et al, 1995). These data suggest opposite site-specific interactions involving CCK and DA, the outcomes of which may differentially impact behavioural responsivity in anxiety-provoking situations.

Central Dopamine Activity and Anxiety-Related Behaviour

It has been suggested that perturbations within the mesocorticolimbic DA system contribute to the behavioural expression of psychopathology, such as schizophrenia (Sesack and Bunney, 1989; Deutch et al, 1990), depression (Anisman and Zacharko, 1990; Bertolucci-D'Angio et al, 1990a; Puglisi-Allegra et al, 1991; Zacharko and Anisman, 1991; Diehl and Gershon, 1992; Rossetti et al, 1993; Brown and Gershon, 1993; Imperato et al, 1993; Tanda et al, 1995) or anxiety (Taylor et al, 1982; Kaneyuki et al, 1991; Simon et al, 1994; Gruen et al, 1995; Beck and Fibiger, 1995; Rodgers et al, 1996). Encounters with aversive life events have been associated with enhanced DA release and metabolism in mesocorticolimbic terminal sites (Herman et al, 1982; Dunn and File, 1983; Abercrombie et al, 1989; Deutch and Roth, 1990; Bertolucci-D'Angio et al, 1990b; Carlson et al, 1991; Heinsbroek et al, 1991; Sorg and Kalivas, 1993). For example, acute restraint selectively increases DA release in the medial prefrontal cortex in rats (Deutch and Cameron, 1992). In

addition, conditioned cue exposure (associated with footshock) enhances DA metabolism in the medial prefrontal cortex, but not in the nucleus accumbens in rats. These data are reminiscent of the observation that mild footshock increased DOPAC in the mesocortex but not the nucleus accumbens in rats (Deutch et al, 1985). A more recent microdialysis study revealed that acute isolation, footshock, or immobilization selectively enhanced DA release in the medial prefrontal cortex in rats (Gresch et al, 1994). Interestingly, the stressful experience of rats witnessing other rats receiving footshock also selectively enhanced mesocortical DA metabolic activity (Kaneyuki et al, 1991).

Electrical stimulation of the medial prefrontal cortex induced ventral tegmental DA cell burst-firing (Gariano and Groves, 1988). Footshock also activates VTA DA cells (Deutch et al, 1985, 1990), in a frequency-dependent manner (Shaham and Stewart, 1994). For example, Shaham and Stewart (1994) reported that very mild footshock increased DA release in the medial prefrontal cortex in rats, compared to Anisman and Zacharko (1986) who observed that a relatively more severe footshock session depleted DA concentrations in this identical cortical site in mice. These data suggest that very mild stressors increase DA release, while more severe stressors induce DA depletions in the medial prefrontal cortex. This position is supported by data demonstrating that mild stressful experiences including exposure to a novel environment (Deutch et al, 1985; Simon et al, 1994), isolation stress (Vezina et al, 1994), mild acute electric shock (Abercrombie et al, 1989) or immobilization (Pei et al, 1990) increase DA neurotransmission preferentially in the mesocortical system in rats. In contrast, repeated protracted restraint (Imperato et al, 1993) or one hour of forced swimming (Rossetti et al, 1993) reduced DA neurotransmission in the medial prefrontal cortex in rats. Although the neural mechanisms underlying DA depletions following more

severe stressful experiences remain to be determined, it will be recalled that GABA_B receptor activation decreases DA release in the mesocortex in rats (Santiago et al, 1993). It is conceivable that mild stressful experiences enhance DA release from DA/CCK terminals in the mesocortex (Shaham and Stewart, 1994), but more severe stressors enhance CCK release. In this respect, CCK induced GABA release in the limbic forebrain (Hughes et al, 1991; Marshall et al, 1991. Ferraro et al, 1996) and cortex in rats (Perez de la Mora et al, 1993), may decrease DA release.

Intrinsic ventral prelimbic and infralimbic interneurons and projection neurons in the medial prefrontal cortex are tonically inhibited by DA from the VTA, (Mantz et al ,1988a, 1988b; Seguela et al, 1988; Sesack and Bunney, 1989; Deutch and Roth, 1990; Deutch et al, 1990; Verney et al, 1990; Godbout et al, 1991; Le Moal and Simon, 1991; Reteaux et al, 1991, 1994; Pirot et al, 1992; Benes et al, 1993; Vincent et al, 1993; Gresch et al, 1994; Sokolowski et al, 1994; King and Finlay, 1995). Accordingly, Mantz et al (1988a) have demonstrated that electrical stimulation of the VTA inhibited the enhanced electrophysiological activity in the medial prefrontal cortex induced by tailshock in rats. Furthermore, the enhanced electrophysiological activity in the medial prefrontal cortex resulting from electrical stimulation of the dorsal medial thalamus, is also inhibited by electrical stimulation of the VTA (Mantz et al, 1988b). Sesack and Bunney (1989) have demonstrated that iontophoretic application of DA in the ventral prelimbic mesocortex hyperpolarize intrinsic colocalized CCK cells, the effects of which were blocked by the D₂ receptor antagonist, sulpiride. These data were supported by the demonstration that DA terminals inhibit intrinsic pyramidal and non-pyramidal cells in the medial prefrontal cortex both directly through D₂ receptors and indirectly through D₁ receptors on GABA

interneurons (Benes et al, 1993; Vezina et al, 1994). In addition, van der Kooy et al (1984) demonstrated that a subset of neurons in the ventral prelimbic mesocortex in rats, which innervates the nucleus of the solitary tract, receive overlapping dorsomedial thalamic and VTA projections. It is noteworthy that, in addition to the nucleus of the solitary tract, the ventral prelimbic and infralimbic areas of the mesocortex in rats project to the basolateral amygdala (Sesack et al, 1989; Takagishi and Chiba, 1991), the central nucleus of the amygdala (Neafsey et al, 1986; Takagishi and Chiba, 1991), the anterior nucleus accumbens (Ladurelle et al, 1995), the VTA and PVN (Sesack et al, 1989; Takagishi and Chiba, 1991), as well as the parabrachial nucleus (van der Kooy et al, 1984) and the rostral ventrolateral medulla (Hurley et al, 1991). It is conceivable that DA depletions in the ventromedial prefrontal cortex produced by stressful situations, or D₂ receptor blockade in this cortical site, could result in enhanced electrophysiological activity in neuronal subsets which project to brainstem nuclei and subcortical nuclei associated with anxiety-related behavioural and neurochemical responsivity to stressful situations. It is noteworthy that electrical stimulation in the posterior ventral prelimbic medial prefrontal cortex induced hypertension and tachycardia in rats (Tucker and Saper, 1984).

Interestingly, restraint-induced DA release in the central nucleus of the amygdala reduced ACTH secretion from the pituitary produced by restraint stress (Beaulieu et al, 1987). In contrast, restraint-induced NE release in the central nucleus of the amygdala stimulated ACTH secretion from the pituitary in rats. These data support the hypothesis of an inhibitory role for DA in the central nucleus of the amygdala in the control of ACTH release in response to stressors (Beaulieu et al, 1987). More recently, lesions of the central nucleus of the amygdala blocked the increase in mesocortical DA activity usually apparent

following exposure to stimuli previously paired with footshock (Davis et al. 1994; Goldstein et al, 1996). Goldstein et al (1996) suggest that the central nucleus of the amygdala may in some way regulate the mesocortical DA system in response to psychological stress. There is also some evidence demonstrating that DA depresses pulmonary function in cats (Wypych and Szereda-Przestaszewska, 1995), as well as blood pressure in rats (Kirouac and Ganguly, 1995). These data, coupled with the inhibitory role of DA on stressor-enhanced medial prefrontal electrophysiological activity (Mantz et al, 1988a), suggest that DA may exert a site-specific anxiolytic profile from this cortical site as well as from the central amygdala.

There are accumulating data suggesting that systemically-administered D₂ or D₃ receptor ligands exert anxiolytic effects in some animal models of anxiety (File, 1996). For example, File (1981) has demonstrated that the D₂ agonist, piribedil, was anxiolytic in the social interaction test in rats. In contrast, low dose systemic administration of the D₂ receptor antagonist, sulpiride, increased punished responding in a conflict test and was anxiolytic in the light/dark test in rats (Pich and Samanin, 1986). These investigators suggested that low-dose systemic neuroleptic administration increased DA transmission from DA terminal sites. Accordingly, a reduction in DA transmission has been suggested to be involved in some aspects of anxiety-related behavioural symptoms (Taylor et al, 1982). Systemic sulpiride was also anxiolytic in the light/dark test and elevated plus maze in mice (Costall et al, 1987; Rogers et al, 1994). Furthermore, the D₂/D₃ receptor agonist, 7-OH-DPAT, was anxiolytic in the elevated plus maze in mice (Rodgers et al, 1996). Moreover, Johnston and File (1989) demonstrated that the anxiogenic effects produced by yohimbine in the elevated plus maze in rats were blocked by the DA receptor agonist, apomorphine. Taken together, these data support a role for DA in reducing anxiety-related behavioural responsivity in some animal

models of anxiety. In this respect, it is noteworthy that microinfused DA decreased stressor-induced NE release in the medial prefrontal cortex in rats (Rossetti et al, 1989). The inhibitory effects of DA on NE release under these circumstances were blocked by the D₂ receptor antagonist, sulpiride. In view of the suggestion that anxiety may stem, at least in part, from an overactive dorsal NE system (Nutt and Lawson, 1992), then the net result of stressor-induced mesocortical DA/dorsal NE interactions in the medial prefrontal cortex could conceivably exert anxiolytic influences on behavioural responsivity.

Central Catecholamine (NE and E) Activity and Anxiety-Related Behaviour

Catecholamine nuclei in the brainstem are activated by interoceptive stimuli, presumably produced by stressful situations, or cues associated with such adversities. For example, Margules and Lichtensteiger (1978) demonstrated that novelty and social isolation increased NE immunofluorescence in the nucleus of the solitary tract in rats. More recently, Pacak et al (1992) demonstrated that immobilization stress increased NE turnover in the PVN in rats. The parvocellular division of the PVN in rats, containing considerable CRF immunoreactivity, is innervated by NE and E fibres from the nucleus of the solitary tract and the ventrolateral medulla (Harbuz et al, 1991). Moreover, the stressful experience of rats exposed to other rats subjected to footshock, enhanced NE turnover in the hypothalamus, the amygdala and the locus coeruleus, as well as plasma corticosterone concentrations (Tanaka et al, 1991). Acute immobilization stress also enhanced NE turnover in the central nucleus of the amygdala (Pacak et al, 1993). The central nucleus of the amygdala in rats receives CA innervation from adrenergic cells, and to a lesser degree from NE cells, originating from the rostral ventrolateral medulla (Zardetto-Smith and Gray, 1995).

In addition to enhanced CA release in the brainstem, the central nucleus of the amygdala and the PVN following novelty, social isolation or restraint, footshock increased NE release in the medial prefrontal cortex from the locus coeruleus in rats (Rossetti et al. 1990). Moreover, Gresch et al (1994) demonstrated that prior exposure to cold could sensitize tailshock-induced NE release in the mesocortex from the locus coeruleus in rats. It is of interest that the locus coeruleus also receives CA innervation from the rostral ventrolateral medulla (Liaw et al, 1992). While it is not clear that stressor-induced NE release in the central nucleus of the amygdala, PVN or mesocortex can be unequivocally connected with enhanced anxiety-like behavioural symptoms, it has been proposed that overactivity of the dorsal NE system could be an etiological factor subserving the behavioural expression of anxiety (Nutt and Lawson, 1992). In this respect, the α_2 adrenoreceptor antagonists, yohimbine and idazoxan, produced panicogenic effects in human subjects with panic disorder, as well as enhanced arousal and produced subjective feelings of anxiety or panic in normal volunteers (Uhde et al, 1989; Albus et al, 1992a, 1992b; Krystal et al, 1992). Likewise in animal studies, Handley and Mithiani (1984b) demonstrated that systemic administration of yohimbine and idazoxan suppressed punished responding, while systemic administration of the α_2 adrenergic agonist, clonidine, increased punished responding in a conflict test in rats. Yohimbine has also been shown to exert anxiogenic effects in the elevated plus maze in rats (Handley and Mithiani, 1984a; Pellow et al, 1985; Johnston and File, 1989; Wada and Fukuda, 1991; Critchley et al, 1992). Interestingly, Johnston and File (1989) observed that the anxiogenic effects produced by yohimbine in the elevated plus maze in rats were blocked by the DA receptor agonist, apomorphine.

In contrast to the anxiogenic effects induced by yohimbine in rats, Cole et al (1995) recently observed that systemic yohimbine produced anxiolytic effects in the elevated plus maze in mice. However, it was suggested that these behavioural effects could be accounted for by yohimbine binding to 5-HT_{1A} receptors, rather than α_2 adrenoreceptor blockade. This conclusion was suggested by the observation that the more selective α_2 antagonist, idazoxan, did not show a significant anxiolytic profile in the elevated plus maze, and clonidine did not block the anxiolytic effects induced by yohimbine in these mice. In addition, DeVry et al (1993) demonstrated that yohimbine and idazoxan were anxiolytic in the ultrasonic vocalization test following footshock in rats, effects which they also suggested were mediated by 5-HT_{1A} receptor activation. In this respect, Gower and Tricklebank (1988) have demonstrated that the 5-HT_{1A} agonist, buspirone, as well as idazoxan and yohimbine, produced anticonflict effects in a shock-induced suppression of drinking test in rats. In this particular situation, the anticonflict effects produced by buspirone were antagonized by clonidine (Gower and Tricklebank, 1988). More convincing evidence in support of 5-HT_{1A} receptor involvement (e.g., anxiolytic effects of yohimbine in the elevated plus maze blocked by 5-HT_{1A} antagonists) is needed before a firm conclusion can be drawn. Nevertheless, numerous data demonstrating that partial 5-HT_{1A} agonists are also potent D₂ receptor agonists suggest that CA and indoleamine receptor ligands may share the same or similar receptor mechanisms in response to stressful environmental events.

Berridge and Dunn (1989a) demonstrated that restraint stress decreased exploratory behaviour in rats. The behavioural deficits induced by restraint were antagonized by clonidine, as well as by the CRF antagonist, α -helical CRF. These investigators suggested that NE mediates the stressor-induced anxiogenic effects on exploratory behaviour through

the stimulated release of CRF. In this respect, microinjected NE in the PVN stimulated CRF mRNA expression in conscious rats (Itoi et al, 1994). Interestingly, electrical stimulation of the PVN in rats elicits tachycardia and increased blood pressure (Pittman and Franklin, 1985; Ter Horst et al, 1991). These data, coupled with evidence of CA innervation of the PVN from the nucleus of the solitary tract and the ventrolateral medulla in the brainstem (Harbuz et al, 1991), position the PVN as a modulator of anxiogenic symptoms produced by CAs, independent of the HPA axis activation.

Central Corticotropin-Releasing Factor Activity and Anxiety-Related Behaviour

Central or peripheral CRF administration is anxiogenic in several animal models of anxiety (Dunn and Berridge, 1990 for review). For example, restraint or intraventricular CRF administration decreased exploratory behaviour in rats (Berridge and Dunn, 1989a) and hypophysectomized mice (Berridge and Dunn, 1989b). Restraint also increased CRF mRNA in the central nucleus of the amygdala and PVN in rats (Kalin et al, 1994). In addition, intraventricular CRF increased plasma epinephrine levels, as well as heart rate and blood pressure in rats (Fisher et al, 1982). Moreover, Liang and Lee (1988) demonstrated that intra-amygdala CRF administration decreased exploratory behaviour in rats. In addition, Heinrichs et al (1992) demonstrated that social defeat enhanced ACTH and GC secretion, and was anxiogenic in the elevated plus maze in rats. The behavioural deficits induced by social defeat in the elevated plus maze could be blocked by microinjecting α -helical CRF in the central nucleus of the amygdala, but α -helical CRF in the central amygdaloid nucleus did not effect the enhanced ACTH and GC secretion induced by social defeat. Heinrichs et al (1992) suggested that CRF produced these anxiogenic effects from the central nucleus of the amygdala independent of the HPA axis activation in rats. Intraventricularly-administered α -

helical CRF was also anxiolytic in the defensive/withdrawal paradigm in rats (Takahashi et al. 1989). Moreover, these investigators observed that rats familiar with the test environment behaved like naive rats following intraventricular CRF administration. Likewise, Yang et al (1990) demonstrated that intraventricular administration of CRF or the α_1 -NE agonist, phenylephrine, potentiated defensive/withdrawal behaviour in rats familiar with the test apparatus. These behavioural deficits were antagonized by α -helical CRF and by the α_1 -NE antagonist, prazosin, respectively, but not by chlordiazepoxide. These data suggest that in some paradigms, anxiety-like behaviour induced by specific anxiogenic agents in CRF-containing sites, is independent of HPA axis activation and not affected by systemic BDZs.

In addition to CRF interactions with NE in the central nucleus of the amygdala or the PVN in rats, restraint stress or intraventricular CRF increased NE and DA release in the medial prefrontal cortex, as well as NE release in the medial hypothalamus in rats (Lavicky and Dunn, 1993; Shimizu et al, 1994). Corticotropin-releasing factor can also stimulate β -endorphin, dynorphin or ACTH release from the PVN in rats (Nikolarakis et al. 1986; Hornby and Piekut, 1989). It is conceivable that CRF-stimulated opioid release in the PVN may provide a neurochemical basis for opioid/HPA interactions, the outcomes of which could impact on the expression of anxiety-related behaviour.

Central Opioid Activity and Anxiety-Related Behaviour

Opioid kappa₁ receptors have been identified in deep layers of the medial prefrontal cortex, the central nucleus of the amygdala, as well as in the nucleus accumbens in rats (Unterwald, Knapp and Zukin, 1991; Mansour et al, 1995). In addition, moderately high levels of the endogenous kappa agonist, dynorphin, are found in these mesocorticolimbic terminal sites (Mansour et al, 1988). Interestingly, dynorphin inhibits spontaneous and

electrically-evoked DA release from terminal sites within these limbic forebrain structures (Olson et al, 1993). It is well known that various stressors activate limbic opioid mechanisms thought to influence some of the emotional aspects of stress (Stein, Hiller and Simon, 1992; Nabeshima et al, 1992; Brandão et al, 1994; Ågmo et al, 1995; Yamada and Nabeshima, 1995). For example, systemic administration of the kappa receptor agonists, U-50,488H or U-69,593, were anxiolytic at low doses but augmented avoidance behaviour in the elevated plus maze at doses higher than 1 mg/kg in rats (Privette and Terrian, 1995). Likewise, systemic U-50,488H induced ultrasonic vocalizations in rat pups reminiscent of those associated with maternal separation (Olson et al, 1993, 1995). U-69,593 or U-50,488H produced conditioned avoidance behaviour following microinjection in the posterior nucleus accumbens in rats (Shippenberg et al, 1993), effects which were coincidental with decreased DA release in this accumbal site (Spanagel et al, 1992). In addition, Bals-Kubik et al (1993) demonstrated that U-50,488H or the dynorphin derivative, E-2078, microinjected in the medial prefrontal cortex also produced conditioned avoidance behaviour in rats. Moreover, systemically- or intraventricularly-administered kappa agonists have been shown to induce hypothermia and inhibit gastrointestinal function in mice (Bansinath et al, 1991).

kappa receptor agonists also decrease locomotor activity in rats under conditioned stressful situations (Yamada and Nabeshima, 1995). More specifically, systemic U-69,593 potentiated conditioned stress-induced immobility, while the kappa antagonist, norBNI, attenuated this anxiety-related behavioural response (Yamada and Nabeshima, 1995). These data are in accordance with microdialysis studies which demonstrated that, in rats placed in an open unfamiliar environment, low-dose subcutaneous U-50,488H did not affect DA release or metabolism in the mesolimbic, nigrostriatal or tuberoinfundibular DA systems

(Donzanti et al. 1992); but higher subcutaneous doses reduced DA release for up to 60 minutes (Donzanti et al, 1992). Interestingly, a 20 minute perfusion of U-50,488H in the nucleus accumbens decreased DA release in rats exposed to the same novel environment (see Donzanti et al, 1992 for discussion). Taken together, these data suggest that dynorphin may influence DA release, at least in part, under stressful conditions (i.e., following activation of the mesolimbic DA system). It is interesting that microinfused U-50,488H decreases DA release in the nucleus accumbens after exposing rats to a novel environment, yet microinfused CCK-8S enhances DA release in the nucleus accumbens in rats following exposure to a novel environment. Thus, whereas CCK-induced mesolimbic DA release following novelty exposure is associated with anxiety (Derrien et al, 1993), U-50,488H-induced mesolimbic DA release inhibition following novelty exposure may also be associated with anxiety. With this in mind, it remains to be determined whether reductions in DA transmission produced by kappa agonists in mesocorticolimbic terminal sites may exert anxiolytic or anxiogenic behavioural effects in animal models of anxiety. It appears likely however that on the one hand if kappa agonists reduce DA transmission affiliated with D₂ receptor systems in the ventromedial prefrontal cortex under stressful environmental conditions, then anxiogenic behavioural effects could follow. On the other hand, if kappa agonists reduce CCK release in stressful situations, then kappa agonists would most-likely exert anxiolytic effects in the mesocortex.

Overview

Despite the evidence suggesting an interactive role for DA, CCK and kappa agonists in the medial prefrontal cortex during anxiety-related behavioural responses to stressful environmental events, it is surprising to note the paucity of data describing the role of this

cortical site in anxiety-related behavioural responsivity in novelty/conflict paradigms such as the elevated plus maze. In view of the reciprocal innervation between the medial prefrontal cortex and brainstem sites in the rat, as well as subcortical areas presumed to influence the behavioural expression of anxiety (Fryszak and Neafsey, 1991, 1994; Owens and Verberne, 1996), the ensuing Experiments evaluated the role of the medial prefrontal cortex and the ventral tegmental area in behavioural responsivity in the elevated plus maze following kappa receptor manipulations. Although the dorsal 5-HT and NE systems as well as the mesocortical DA system, which innervate the medial prefrontal cortex, are readily activated in response to threatening environmental stimuli (Goldstein, Rasmusson, Bunney and Roth, 1996), there are currently no data which describe the influence of kappa receptor ligands on elevated plus maze performance following intracerebral administration.

The present series of Experiments assessed a) the effects of individual and group housing conditions on subsequent elevated plus maze performance, b) the behavioural effects of the κ_1 receptor agonist, U-69,593, and the kappa antagonist, nor-BNI, in the elevated plus maze following microinjection in the medial prefrontal cortex in CD-1 mice and (c) the behavioural effects of U-69,593 and norBNI in the elevated plus maze following microinjection in the VTA.

Experiment 1

Some laboratories have demonstrated that socially isolated rats exhibit more anxiety-related behaviour in the elevated plus maze relative to group-housed animals (e.g., Motta et al, 1992; Maisonneuve et al, 1993; Vasar et al, 1993b). Vasar et al (1993b), for example, demonstrated that the increased anxiety evident among isolated rats in the elevated plus maze

was correlated with enhanced CCK_B receptor density in the prefrontal cortex. In contrast to the anxiogenic effects of social isolation in the elevated plus maze in rats, social isolation produced anxiolytic effects in the elevated plus maze in male mice (Hilakivi and Lister, 1989; Rodgers and Cole, 1993b). The differential effects produced by variable housing conditions between rats and mice are reminiscent of variable behavioural effects produced by high illumination in the elevated plus maze in these same species. In particular, whereas increased illumination has been shown to have little effect on plus maze behaviour in rats (Baldwin and File, 1986; Falter et al, 1992; Handley and McBlane, 1993) illumination intensity appears to exert anxiogenic effects in CD-1 mice (preliminary data, this laboratory). Accordingly, preliminary trials were also conducted to identify the testing particulars which promoted an anxiogenic condition while concomitantly permitted detection of graded increases or decreases in performance from baseline measures. Taken together, Experiment 1 was designed to evaluate the behavioural effects of individual and group housing in the elevated plus maze in male CD-1 mice in a mildly anxiogenic challenge condition.

Materials and Methods

Subjects. Naive, male, CD-1 mice were employed as subjects. The mice were obtained from Charles River (St. Constant, Quebec) at approximately 5 weeks of age. All mice were initially housed in groups of 4 in polypropylene cages in a temperature controlled room ($21 \pm 1^\circ \text{C}$), and permitted ad libitum access to food and water. Mice were acclimatized for 6 to 8 weeks and achieved a weight range of 36-46 grams prior to the Experiment. All mice were maintained on a 12 hour light/dark cycle (lights on at 7 a.m.).

Apparatus. The elevated plus maze employed in the present Experiments is a modified version of the apparatus described by Lister (1987). The two opposing closed arm runways

were 30 cm x 5 cm and enclosed by 15 cm high walls on each side and the enclosed ends of the apparatus. The two opposing open arms were also 30 cm x 5 cm with a .25 cm high Plexiglas edge on either side and the end of each runway. The centre platform was 5 cm x 5 cm. The enclosed arm walls were constructed with urethane-coated wood and lined with Plexiglas. The floor of the maze was covered with a thin layer of vulcanized black rubber to facilitate intertrial cleaning of the apparatus. The apparatus was positioned 40 cm from the floor on a wooden stand (see Plate 1).

Plate 1. Photograph depicting the elevated plus maze used in Experiments 1 and 2. Note the black background and floor under the stand.

Procedure. Mice were randomly assigned to either (a) a group housed condition ($n = 8$) consisting of 4 mice per cage or (b) a socially-isolated condition ($n = 8$) with one mouse per cage. The subjects in the isolated condition were singly housed for 3 weeks prior to behavioural testing. Following completion of the isolation period of the singly housed animals, mice from either condition were transferred from the main animal holding area to the laboratory and left undisturbed for at least 1 hour. Individual trials were 5 minutes in duration and commenced following placement of the animal in the central platform facing

an open arm (Derrien et al, 1993; Privette and Terrian, 1995). The floor and Plexiglas walls were wiped between trials with a 70% ETOH/dH₂O solution to prevent individual mice from following the scent of a previously tested animal (Privette and Terrian, 1995). All testing was conducted during the mid-morning phase of the light/dark cycle between the hours of 8 a.m. and 12 p.m. A black curtain suspended from ceiling to floor enclosed the elevated plus maze to provide a test chamber approximately 2 m x 2 m within the test room. Dim light conditions were provided by reflecting the light from a directional desk lamp (situated outside the curtained test area) off the ceiling tiles and into the test chamber. All trials were recorded by a vertically mounted Sony CCD-TR4 video camera/VCR (set up on an HD Vivitar Tripod in the enclosed test area) that was linked to a Panasonic video monitor positioned outside the test chamber. A mouse was considered to have entered into or exited an arm when all 4 paws were positioned either within or out of an arm runway respectively (Lister, 1987; Andrews and File, 1993; Derrien et al, 1993). Behaviour in the elevated plus maze was categorized into factors related to anxiety, locomotor activity, exploration and conflict/decision activity, identified in several factor analytic studies for both mice and rats (Trullas and Skolnick, 1993; Rodgers and Johnson, 1995; Cruz et al, 1994; Fernandes and File, 1996). For the purposes of the present study, Factor 1 refers to anxiety related behaviour reported as the number of open arm entries (OE), the open arm entry ratio (open arm entries ÷ total arm entries x 100 -- OER), the open arm time ratio (open arm time ÷ total time on the maze -- OTR), the closed arm time ratio (closed arm time ÷ total time on the maze -- CTR), the ratio of head dips from a protected area (protected head dips ÷ protected + unprotected -- PHDR) and the ratio of stretch attend postures from a protected area (protected stretch attends ÷ protected + unprotected -- PSAR) (see Plate 2).

In addition, a novel measure will be included in Factor 1 referred to as the mean time (sec) per open arm visit (MOVT). Preliminary data from this laboratory revealed that anxiolytic treatments increase and anxiogenic treatments decrease this temporal measure. An increase in OE, OTR or OER is considered to be an anxiolytic index of treatment (Lister,

Plate 2. Photograph depicting a CD-1 mouse engaging in a) a stretch attend posture from the centre hub into an open arm of the maze and b) an unprotected head dip from an open arm of the maze.

1987; Cole et al 1995, Fernandes and File, 1996), as is a decrease in PHDR and PSAR (Rodgers and Johnson, 1995). An increase or decrease in the ratio of time spent in the centre hub (time in central hub ÷ total time -- HTR) was used as an index of conflict (decision) resolution (Factor 2) in Experiment 1 (Rodgers and Johnson, 1995). The time spent in the centre hub has been defined as an index of infrahuman decision making stemming from approach/avoidance conflict (Trullas and Skolnick, 1993; Cruz, Frei and Graeff, 1994; Rodgers and Johnson, 1995; Fernandes and File, 1996). Total arm entries (TE) and the number of closed arm entries (CE) have been employed as indices of locomotor activity (Factor 3) (Lister, 1987; Belzung and Le Pape, 1994; Fernandes and File, 1996; but see Discussion in Experiment 2). Locomotor activity-induced artifacts are partially controlled for by measuring the OER (Hogg, 1996), but CE is considered by some investigators to be

a more appropriate measure of locomotor activity since this behaviour can vary independently of the OER (File, 1992). The cumulative number of head dips (THD Factor 4) was employed as an index of an animal's tendency to explore the maze (Cruz, Frei and Graeff, 1994; Rodgers and Johnson, 1995). Anxiolytic drugs typically increase or have no effect on THD, whereas anxiogenic drugs ordinarily decrease this behaviour (Cruz et al, 1994; Rodgers, Cole and Davies, 1994; Cole and Rodgers, 1994).

Statistical Analyses. The behavioural data describing OE, OER, OTR, CTR, MOV, PHDR and PSAR, as well as TE, CE, THD and HTR were analyzed following a priori predictions by 1-tailed independent samples t-tests for differences between group means ($\alpha = .025$), thus reducing the probability of Type 1 errors due to repeated testing of the same animal. Levene's adjustment of df 's was also employed when unequal variances were detected.

Results

The results and associated t-test data for Experiment 1 are summarized in Table 1.

Behaviour	Group Housed	Single Housed	$t_{(df)}^*$	1-tailed p
OE	3.5 $\pm$.6	6.5 $\pm$ 1.6	$t_{(8.88)} = 1.74$	.05 NS
OER	.211 $\pm$.035	.312 $\pm$.060	$t_{(11.24)} = 1.46$	.09 NS
OTR	.113 $\pm$.019	.214 $\pm$.048	$t_{(9.12)} = 1.95$	.04 NS
MOV	8.7 $\pm$ 1.5	10.6 $\pm$ 1.2	$t_{(13.52)} = 1.01$	.17 NS
CTR	.318 $\pm$.024	.314 $\pm$.030	$t_{(13.36)} = .09$	.46 NS
PHDR	.638 $\pm$.063	.471 $\pm$.104	$t_{(11.5)} = 1.38$	.10 NS
PSAR	.737 $\pm$.047	.690 $\pm$.058	$t_{(13.4)} = .62$	.28 NS
TE	16.6 $\pm$.8	20.1 $\pm$ 1.9	$t_{(9.3)} = 1.71$	.05 NS
CE	13.1 $\pm$.8	13.6 $\pm$ 1.5	$t_{(10.93)} = .30$	.39 NS
THD	16.5 $\pm$ 2.7	25.1 $\pm$ 6.8	$t_{(9.24)} = 1.18$	.13 NS
HTR	.569 $\pm$.016	.472 $\pm$.032	$t_{(10.4)} = 2.71$	< .025 **

Table 1. Values = mean $\pm$ SEM. Time Ratios represent the amount of time (sec) on a section $\div$ total time (300 sec). TE, total arm entries; OE, open arm entries; OER, open arm entry ratio; OTR, open arm time ratio; MOV, mean time (sec) per open arm visit; CE, closed arm entries; CTR, closed arm time ratio; HTR, centre hub time ratio; THD, total head dips; PHDR, protected head dip ratio; PSAR, protected stretch attend ratio. (df 's* were adjusted because of unequal variances between groups. 1-tailed t -tests with reduced $\alpha = .025$ to counteract increased probability of Type 1 errors stemming from repeated t -tests on the same subject. ** $p < .025$).

Independent samples t-tests revealed that 3 weeks of social isolation reduced the ratio of time

spent in the centre hub ($t_{10.4} = 2.71, p < .025$) of the elevated plus maze (see Fig. 1).

Although the ratio of time spent in the open arms was increased ($t_{9.12} = 1.95, p = .04$ NS), this effect did not reach an acceptable level of statistical significance (see Fig. 2). The open

arm entry ratio, closed arm time ratio, as well as the average time spent per open arm visit were not altered by isolation housing (see Table 1). In addition to the failure of isolation to influence traditional measures of anxiety, social isolation likewise did not influence ethological measures of anxiety as revealed by the protected head dipping and protected stretch attend ratios (see Table 1). Finally, individual housing failed to effect locomotor activity as revealed by total and closed arm entries, as well as exploration activity as measured by total head dips (see Table 1).

Discussion

Three weeks of social isolation did not alter traditional anxiety measures operationally defined by OE, OER, OTR and CTR in Experiment 1. These data are consistent with results derived from another laboratory showing similar lack of effects on anxiety related time-dependent ratios in the elevated plus maze in male DBA/2 mice following 3 weeks of social isolation (Rodgers and Cole, 1993a). In contrast, Hilakivi, Ota and Lister (1989) demonstrated that 20 days of social isolation significantly increased the OER and OTR in male NIH Swiss mice relative to group housed animals. Although the OTR was increased in CD-1 mice in Experiment 1, the variability among isolated mice likely prevented this measure from reaching an acceptable level of significance. In any event, it is of interest that isolation housing was not anxiogenic in DBA/2, NIH Swiss or CD-1 mice, particularly regarding anxiety-related time-dependent behaviours.

In addition to the paucity of effects on traditional anxiety measures employed in the present study, isolation housing also failed to alter ethologically-derived anxiety-related exploration and risk assessment behaviours (i.e., the proportion of these behaviours performed in a protected area of the maze, PHDR or PSAR). These data differ from Rodgers

and Cole (1993a) where 3 weeks of isolation had no effect on the PHDR, but reduced the PSAR in male DBA/2 mice. Taken together, although isolation housing produced an anxiolytic behavioural profile in male NIH Swiss mice (Hilakivi et al, 1989), housing condition was ineffective in influencing traditional Factor 1 anxiety-related indices evaluated in Experiment 1. In addition, isolation housing was anxiolytic in male DBA/2 mice with respect to protected risk assessment behaviour (Rodgers and Cole, 1993a), but had no effect on these measures in male CD-1 mice relative to group housed animals.

It would appear at first glance that isolation increased locomotor activity (i.e., significantly increased TE) in NIH Swiss mice (Hilakivi et al, 1989), but had no effect on TE in DBA/2 (Rodgers and Cole, 1993a) or CD-1 mice in the present study. These apparent strain differences in locomotor activity can possibly be explained by the low basal level of total arm entries evident among NIH Swiss control mice relative to either DBA/2 or CD-1 mice. Although TE are considered to be an index of locomotor activity (e.g., Lister, 1987), TE also encompasses some aspects of anxiety-related behaviour (Hogg, 1996). In this respect it is noteworthy that CE were not increased in DBA/2, NIH Swiss or CD-1 mice. In effect, increased total arm entries evident among NIH Swiss mice (Hilakivi et al, 1989) may well reflect an anxiolytic consequence of isolation, rather than a stimulatory effect on locomotor activity per se.

The spatiotemporal distribution of section preference (i.e., open arms vs. closed arms vs. centre hub) in the elevated plus maze evident among DBA/2 mice differed from that observed in CD-1 mice in Experiment 1. Whereas DBA/2 mice from either housing condition preferred the closed arms > centre hub > open arms (Rodgers and Cole, 1993a), group housed CD-1 mice preferred the centre hub > closed arms > open arms in the present

study. Furthermore, although isolation housing significantly reduced the HTR in CD-1 mice in Experiment 1, the reduced percentage of time spent in the centre platform by CD-1 isolates was still considerably higher than that observed by Rodgers and Cole (1993a). It would appear that CD-1 mice may be influenced by the conflicting nature of the elevated plus maze to a greater degree than DBA/2 mice, thus remain indecisive in the centre hub for longer durations presumably engaging in protected risk assessment behaviour. One possible explanation for the discrepant HTR scores between DBA/2 and CD-1 mice may lie within some methodological differences between the two laboratories. Whereas DBA/2 mice in Rodgers and Coles' (1993a) study were maintained on a reverse light/dark cycle and tested during the dark phase, CD-1 mice in Experiment 1 were maintained on a normal light/dark cycle and tested during the light phase. In addition, the closed arms employed by Rodgers and Cole (1993a) were constructed of transparent Plexiglas as opposed to Plexiglas-lined wood in the apparatus in this laboratory. In view of the observation that DBA/2 mice are an inbred strain and CD-1 mice are an outbred strain may also account for some of the apparent behavioural differences evident between the mouse strains.

Taken together, there were no statistically significant between groups differences in anxiety-related behaviour owing to housing manipulations evident among the CD-1 mice observed in Experiment 1. In fact, the only behavioural measure significantly reduced in CD-1 mice was the conflict/decision components of the elevated plus maze (Factor 2). These results indicate that variable housing does not substantially alter elevated plus maze behavioural profiles in male CD-1 mice. The present findings a) confirm that the construction of the maze, illumination levels and test chamber conditions employed, as

derived from preliminary trials, are suitable for ensuing Experiments and b) lend support to the use of isolated mice following surgery in subsequent Experiments.

Experiment 2

Several lines of evidence suggest that there are some neuronal regions in the medial prefrontal cortex which innervate the nucleus of the solitary tract (Takagishi and Chiba, 1991) and the rostral ventrolateral medulla in the brainstem in rats (Dampney et al, 1982). It has been suggested that these neuronal sub-populations are under tonic inhibition by DA from the VTA (van der Kooy et al, 1984; Sesack et al, 1989; Pirot et al, 1992). This intrinsic mesocortical neuronal sub-population can also be electrophysiologically enhanced by CCK release, at least in part, from the VTA where CCK may coexist with DA in some of the same cell bodies of origin in the mesocortical system, as well as from the dorsomedial nucleus of the thalamus, the basolateral amygdala (Hökfelt et al, 1985) or from cortical CCK/GABA-containing interneurons (Jones and Hendry, 1986). Electrical stimulation of the ventromedial prefrontal cortex influences blood pressure, heart rate, respiration, gastric motility, urination and regional blood flow (Frysztyk and Neafsey, 1994). Accordingly, it has been proposed that the ventromedial prefrontal cortex may be a visceromotor centre involved in the expression of autonomic responses to stressful or emotional stimuli, as well as in the expression of visceral emotional responses to conditioned stimuli (Owens and Verberne, 1996). There is some evidence to suggest that CCK_B receptor activation in the medial prefrontal cortex in rats and mice is correlated with anxiety-related behaviour in the elevated plus maze (Harro et al, 1990a, 1990b, 1990c; Harro and Vasar, 1991; Harro, Vasar and Bradwejn, 1993 for review). Likewise, CCK_B receptor binding is also enhanced in the rat prefrontal cortex following anxiogenic drug treatment, exposure to novel environments and

social isolation (Harro et al, 1990a, 1990b; Vasar et al, 1993b). More recently, Harro et al (1996) have demonstrated that CCK_B receptor binding is enhanced in the frontal cortex in rats following exposure to the sounds and odor of other rats undergoing decapitation. Similarly, it will be recalled that DA metabolic activity is enhanced in the medial prefrontal cortex following a variety of stressful stimuli in rats and mice (e.g., Pei et al, 1990), including exposure to other rats receiving footshock (Kaneyuki et al, 1991). In view of the observation that DA and CCK activity is enhanced in the medial prefrontal cortex under anxiety-provoking environmental conditions, it could be suggested that the mesocortical DA/CCK terminals in the medial prefrontal cortex may be involved in behavioural responses to anxiogenic environments such as the elevated plus maze.

It is also worth noting that neuropeptides within the mesocortical system which interact with DA are only activated following DA activation (Crawley, 1991; Donzanti et al, 1992). In this respect, kappa receptor agonists have been shown to decrease basal, electrically-evoked and drug-induced DA release in mesolimbic, nigrostriatal, mesocortical and tuberinfundibular terminal fields (Werling et al, 1988; Manzanares et al, 1991, Donzanti et al, 1992; Shippenberg et al, 1993; Olson et al, 1993, 1995). The behavioural implications of such DA/dynorphin interactions have for the most part not been investigated. There are some data, however, demonstrating that microinfusion of the non-selective κ agonists, U-50,488H or E-2078, in the medial prefrontal cortex produces conditioned avoidance behaviour in rats concomitant with decreased DA release (Bals-Kubic et al, 1993). In this regard, dynorphin is colocalized in GABA-containing interneurons in the medial prefrontal cortex, but is absent in afferent cortical fibres in most species (Jones & Hendry, 1986; Mansour et al, 1988). It should be noted however that a) conditioned avoidance paradigms

may or may not involve anxiety-related behaviour, b) U-50,488H and E-2078 were administered under non-stressful basal conditions (Bals-Kubik et al, 1993), and c) non-selective κ agonists could be producing behavioural effects at either κ_1 or κ_2 receptor sites, which have been detected in moderate density in deep layer VI of the medial prefrontal cortex in rats (Unterwald, Knapp and Zukin, 1991). In this respect, opioid receptor agonists which enhance DA release in the mesocorticolimbic system do not produce conditioned avoidance behaviour, whereas kappa receptor agonists that reduce DA release in the mesocorticolimbic system produce conditioned avoidance in rats (Spanagel, Herz and Shippenberg, 1992). In addition, drugs which enhance DA release also enhance dynorphin release in mesocortical terminal areas (Smiley et al, 1990). The suggestion that the behavioural avoidance produced by U-50,488H in the medial prefrontal cortex under basal environmental conditions was likely due to DA release inhibition (Bals-Kubik et al, 1993) is intriguing. Indeed, behavioural data available from several laboratories have revealed that exposure of rats to the elevated plus maze enhances DA and CCK release, as well as CCK_B receptor binding in the medial prefrontal cortex (Harro and Vasar, 1991). Furthermore, it has been argued that the most salient expression of anxiety-related behaviour in the elevated plus maze is unconditioned overt avoidance of open arm activity (Rodgers and Johnson, 1995). In this respect, since enhanced CCK receptor density and binding in the medial prefrontal cortex is correlated with such anxiety-related behaviour, pharmacological manipulations that can reduce CCK release in this cortical site should exert anxiolytic effects on behaviour.

Experiment 2 was designed to examine the possibility that the selective κ_1 agonist, U-69,593, microinjected in the medial prefrontal cortex, would dose-dependently induce an anxiolytic influence in the elevated plus maze. The rationale for this study was predicated

on the prediction that κ_1 receptor activation on mixed DA/CCK terminals (Mansour et al, 1995) would decrease CCK release in the medial prefrontal cortex in CD-1 mice. Low dose U-69,593 administration in the medial prefrontal cortex should presumably decrease CCK release and exert anxiolytic effects, whereas higher drug concentrations should also decrease DA release and provide reduced anxiolytic influences.

It should be underscored that the demonstration of anxiolytic effects in the elevated plus maze following U-69,593 administration in the medial prefrontal cortex would only suggest that the mesocortical DA/CCK system interacts with kappa opioid receptors in behavioural responses to anxiogenic environments. Indeed, such data would not only implicate mesocortical DA/CCK terminal fields on the expression of anxiety in the elevated plus maze, but also potentially suggest an involvement of the DA/CCK cell dendritic fields in the VTA. In this respect, it will be recalled that medial prefrontal interneurons are inhibited by microiontophoretically applied DA (Sesack and Bunney, 1989), as well as by electrical stimulation of DA cells in the VTA (Thierry et al, 1986). Furthermore, projections from the caudal region of the ventral prelimbic medial prefrontal cortex form excitatory synapses on DA cells in the VTA (Sesack, Deutch, Roth and Bunney, 1989; Sesack and Pickel, 1992). Moreover, some physiological studies have demonstrated that electrical stimulation of the ventral medial prefrontal cortex enhanced the electrophysiological activity of DA neurons in the VTA (Thierry et al, 1979; Gariano and Groves, 1988). Accordingly, disruption of the mesocortical DA innervation from the VTA results in an enhanced subcortical mesolimbic DA response to stressful environmental events in rats (Deutch, Clark and Roth, 1990). In view of the aforementioned data, U-69,593 was also microinjected in the VTA to address the site-specific nature of this kappa receptor agonist. There are some data

which suggest that direct application of κ agonists in rat VTA cell cultures fails to alter the electrophysiological activity of DA cells (Johnson and North, 1992). Likewise, microinjection of U-69,593 in the VTA failed to modify DA release in rats (see Spanagel, 1995 for review). Moreover, Bertolucci-D'Angio et al (1990a) interestingly demonstrated that the increase in mesocortical DA metabolism induced by novelty, loud noises or restraint in rats was not connected to the emotional reactive state brought on by the aversive nature of these particular stressors. In any event, it should be underscored that there are few κ_1 receptor binding sites in the VTA in rats (Mansour et al, 1995). Taken together, U-69,593 would not be expected to provoke alterations in anxiety-related behaviour in the elevated plus maze following microinjection in the VTA in CD-1 mice.

Nor-binaltorphamine (norBNI) is a selective kappa opioid receptor antagonist in mice (Portoghese et al, 1987). Since kappa₁ receptors in the medial prefrontal cortex are expressed on DA/CCK terminals (Mansour et al, 1995), norBNI should enhance CCK release in this cortical site in response to elevated plus maze exposure. In this respect, the hypothesis was entertained that norBNI, microinjected in the medial prefrontal cortex, would induce a dose-dependent anxiogenic influence in the elevated plus maze in Experiment 2. In addition, since DA/CCK cell bodies in the VTA are largely devoid of κ_1 receptors (Mansour et al, 1988), norBNI was also microinjected in the VTA as a comparison site. It was expected that norBNI would exert little, if any, effect on behaviour in the elevated plus maze following microinjection in the VTA.

Materials and Methods

Subjects. Naive, male, CD-1 mice were used as subjects. The mice were obtained from Charles River (St. Constant, Quebec) at approximately 5 weeks of age. All mice were

initially housed in groups of 4 in polypropylene cages in a temperature controlled room ($21 \pm 1^\circ \text{C}$), and permitted ad libitum access to food and water. Mice were acclimatized for 6 to 8 weeks and achieved a weight range of 36–46 grams prior to the Experiment. All mice were maintained on a 12 hour light/dark cycle (lights on at 7 a.m.).

Apparatus. The particulars pertaining to elevated plus maze specifications and construction were identical to those described in Experiment 1.

Surgery. Mice were anesthetized with an anaesthetic cocktail consisting of sodium pentobarbitol (0.65 mg/ml), diazepam (5 mg/ml) and saline (0.9%) administered i.p. at doses ranging between 0.5 and 0.75 ml/100 g. A 23 gauge stainless steel guide cannula approximately 1.1 cm in length was unilaterally implanted in the medial prefrontal cortex or the VTA using a David Kopf micromanipulator. All cannulae were either implanted in the medial prefrontal cortex employing the following stereotaxic coordinates: A.P. +2.0 mm anterior to Bregma, L. +0.4 mm from the midsagittal suture and V. -2.0 mm from a flat skull surface, or the VTA using the following coordinates: A.P. -3.5 mm posterior to Bregma, L. +0.4 mm from the midsagittal suture and V. -4.5 mm from a flat skull surface. Postoperatively, mice were housed individually in polypropylene cages on warm heating pads for at least 7 days and supplemented with a wet mash derived from ground lab chow and a high protein Meritene mixture. Mice were allowed a 2 week recovery period in the main animal housing room prior to experimentation.

Drugs. Nor-BNI and U-69,593 were obtained from Research Biochemicals International, Natick, MA. Nor-BNI was dissolved and mixed with saline to concentrations of 1 nmol/ μl

and 10 nmol/ μ l. U-69,593 was dissolved and mixed with saline to concentrations of 10 nmol/ μ l and 100 nmol/ μ l. NaCl was dissolved in dH₂O (0.9 % concentration).

Intracerebral drug administration. Following a 2 week recovery period, mice were transferred from the main holding area to the laboratory and left undisturbed for 1 hour prior to intracerebral drug administration (Rogers et al, 1995). In Experiment 2, mice were randomly assigned to a 0 nmol vehicle, a 10 nmol U-69,593, a 100 nmol U-69,593, a 1 nmol norBNI or a 10 nmol norBNI treatment condition and challenged with either the agonist or the antagonist in the medial prefrontal cortex (n = 8/cell). Additional mice were randomly assigned to a 10 nmol U-69,593 or a 10 nmol norBNI treatment condition and challenged with either κ_1 ligand in the VTA. Each mouse was lightly restrained and a 30 gauge injection cannula was inserted into the guide cannula. The injection cannula was connected via polyethylene tubing to a 5 μ l Hamilton microsyringe. Each drug concentration was infused in a 1 μ l volume over a 60 second period. Injection cannulae remained in place for an additional 60 seconds to allow drug diffusion.

Procedure. Immediately following drug infusion, each animal was returned to its home cage, brought into the testing room and left undisturbed for 5 minutes prior to behavioural evaluation. Each mouse participated in a single 5 minute trial which commenced following placement of the animal in the central platform facing an open arm (Derrien et al, 1993; Privette and Terrian, 1995). Each trial was conducted and monitored as described in Experiment 1.

Histology. Mice were sacrificed with an overdose of sodium pentobarbitol and perfused intracardially with 0.9% saline followed by a 10% formalin solution. Mice were decapitated, the head caps removed, brains excised from the cranial cavity and stored in formalin for at

extent of the cannula placement. Brain sections were mounted, stained with cresyl violet and examined under a microscope. The distribution of cannula placements in the medial prefrontal cortex and the VTA were mapped on schematic representations of the respective brain sites employing the mouse brain atlas of Franklin and Paxinos (1997).

Statistical Analyses. The data describing anxiety related behaviours (open arm entries, open arm entry ratio, open arm time ratio, mean time per open arm visit, closed arm time ratio, protected head dip ratio and protected stretch attend ratio), as well as locomotor activity scores (total entries and closed entries), exploration (total head dips) and conflict/decision measures (centre hub time ratio) in the elevated plus maze were analyzed by a one-way analysis of variance (ANOVA) for dose response analyses from the medial prefrontal cortex. Where indicated by significant main effects ($\alpha = .05$), post hoc 1-tailed independent samples t-tests were conducted to test a priori unidirectional predictions following Levene's *df* adjustment for unequal variances between groups for comparisons of treatment group means with control group means ($\alpha = .025$). Data from the VTA were analyzed a) by 1-tailed independent samples t-tests to compare the U-69,593 group means with the norBNI group means ($\alpha = .025$) and b) 1-tailed independent samples t-tests were conducted to compare the 10 nmol U69,593 medial prefrontal and VTA groups with the vehicle group, followed by the 10 nmol norBNI medial prefrontal and VTA groups with the vehicle group ($\alpha = .025$). Levene's *df* adjustment was also employed when unequal variances between groups were detected.

Results

U-69,593 in the medial prefrontal cortex. The array of cannula placements among mice challenged with vehicle, 10 or 100 nmol U-69,593 subjects in the medial prefrontal cortex are depicted in Plate 3.

Plate 3. Cannula placements for the 0 nmol, 10 nmol and 100 nmol U-69,593 subjects in the medial prefrontal cortex. All placements were histologically verified from 40 μ m mounted brain slices stained with cresyl violet.

Only those mice with correctly placed cannulae in the medial prefrontal cortex within the rostral-caudal range defined as +1.5 mm to +2.2 mm anterior to Bregma, the dorsal-ventral range defined as -1 mm to -2.2 mm ventral to a flat skull surface and the medial-lateral range defined as +0.2 mm to +1.4 mm lateral to the midsagittal suture were included in the present analysis (mouse brain atlas of Franklin & Paxinos, 1997). Three mice from the saline group, 4 from the 10 nmol and 2 from the 100 nmol groups were excluded due to placements outside the aforementioned criteria.

Plate 4. Photomicrographs of coronal section Bregma + 2.1 mm depicting representative cannulae placements in the medial prefrontal cortex for A) subject # 7 from the 10 nmol U-69,593 group and B) subject # 3 from the saline control group.

The behavioural data and associated ANOVA results pertaining to κ_1 agonist administration in the medial prefrontal cortex are summarized in Table 2. Analysis of variance of OE, OER or OTR following intracerebral U-69, 593 administration failed to reveal significant main effects from the medial prefrontal cortex ($F_{2,21} = .039, .834$ and

U-69,593 in the Medial Prefrontal Cortex

Behaviour	0 nmol	10 nmol	100 nmol	<i>F</i> (2, 21)	<i>p</i>
OE	8.4 ± 1.2	8.4 ± 1.2	7.9 ± 1.9	.04,	<i>p</i> = .96
OER	.276 ± .04	.370 ± .058	.309 ± .059	.83,	<i>p</i> = .45
OTR	.217 ± .022	.348 ± .050 **	.282 ± .061	1.97,	<i>p</i> = .17
MOVT	8.1 ± .4	12.5 ± .4 ‡	11.6 ± 1.0 †	12.61,	<i>p</i> < .001
CTR	.340 ± .035	.306 ± .038	.380 ± .047	.85,	<i>p</i> = .44
PHDR	.354 ± .031	.321 ± .049	.271 ± .046	.97,	<i>p</i> = .40
PSAR	.537 ± .045	.280 ± .075 **	.423 ± .067	4.10,	<i>p</i> < .05
TE	31.0 ± 2.1	23.5 ± 1.1 †	24.1 ± 1.8 **	5.74,	<i>p</i> < .01
CE	22.6 ± 2.2	15.1 ± 2.1 **	16.3 ± 1.3 **	4.64,	<i>p</i> < .025
THD	27.8 ± 3.5	34.0 ± 3.9	28.3 ± 6.2	.55,	<i>p</i> = .58
HTR	.443 ± .028	.337 ± .026 **	.337 ± .024 **	5.55,	<i>p</i> < .025

Table 2. Values = mean ± SEM. Time ratios represent the amount of time (sec) on a section ÷ total time (300 sec). TE, total arm entries; OE, open entries; OER, percent open arm entries; OTR, open arm time ratio; MOVT, mean time (sec) per open arm visit; CE, closed entries; CTR, closed arm time ratio; HTR, centre hub time ratio; THD, total head dips; PHDR, protected head dip ratio; PSAR, protected stretch attend ratio. Following oneway ANOVA, treatment group means were compared with vehicle group means. (Significant 1-tailed difference from control group: ** *p* < .025, † *p* < .01, § *p* < .005, ‡ *p* < .000 after Levene's *df* adjustment for unequal variances between groups).

1.969, NS) respectively. It should be noted parenthetically that the 10 nmol U-69,593 concentration reduced the OTR when first tested by a 1-tailed t-test. Adding the 100 nmol

group however resulted in no main effect of treatment on this measure (see Fig. 3). U-69,593 administration significantly increased the MOVT ($F_{2,21} = 12.61$, $p < .001$). Post hoc comparisons revealed that both the 10 nmol ($p < .0000$) and 100 nmol ($p < .01$) U 69,593 concentrations increased the MOVT relative to saline controls (see Fig. 4). The CTR and the PHDR were also not reduced by U-69,593 ($F_{2,21} = .852$ and $.968$) respectively (see table 2).

however, the percentage of stretch attend postures performed from protected areas of the maze was ($F_{2,21} = 4.097$, $p < .05$). A post hoc 1-tailed t-test revealed that the 10 nmol U-69,593 concentration decreased the PSAR ($p < .025$) relative to vehicle-treated mice (see Fig. 5). U-69,593 also reduced locomotor activity as measured by the number of total arm entries ($F_{2,21} = 5.74$, $p < .01$) and closed arm entries ($F_{2,21} = 4.641$, $p < .025$). Both the 10 nmol ($p < .01$) and 100 nmol ($p < .025$) U-69,593 concentrations reduced total arm entries

compared with the saline group (see Fig. 6). Likewise, independent samples t-tests revealed

that both the 10 nmol ($p < .025$) and 100 nmol ($p < .025$) drug concentrations reduced the

number of closed arm entries relative to controls (see Fig. 7). One-way ANOVA revealed

that exploratory activity (THD) was not effected by either drug concentration ($F_{2,21} = .551$, NS). The percentage of time spent in the centre hub was reduced by U-69,593 ($F_{2,21} = 5.553$, $p < .025$). Subsequent post-hoc comparisons revealed that both the 10 nmol ($p < .025$) and 100 nmol ($p < .025$) U-69,593 concentrations reduced this index relative to the vehicle control group (see Fig. 8).

NorBNI in the medial prefrontal cortex. The array of cannula placements for the 1 nmol and 10 nmol norBNI subjects in the medial prefrontal cortex are depicted in Plate 5. Mice with properly placed cannulae as described previously were included in the current analysis.

Plate 5. Cannula placements for the 1 nmol and 10 nmol norBNI subjects in the medial prefrontal cortex. All placements were histologically verified from 40 μ m mounted brain slices stained with cresyl violet.

Plate 6 depicts a correct cannula placement in the medial prefrontal cortex for subject # 4 from the 10 nmol norBNI group.

Plate 6. Photomicrograph of coronal section Bregma + 2.1 mm depicting a representative cannula placement in the medial prefrontal cortex for subject # 4 from the 10 nmol norBNI group.

The behavioural data and associated ANOVA results pertaining to kappa antagonist administration in the medial prefrontal cortex are summarized in Table 3. One-way ANOVA

norBNI in the Medial Prefrontal Cortex					
Behaviour	0 nmol	1 nmol	10 nmol	<i>F</i> (2, 21)	<i>p</i>
OE	8.4 ± 1.2	7.6 ± 1.2	3.6 ± 1.1 †	5.03,	<i>p</i> < .025
OER	.276 ± .036	.255 ± .041	.147 ± .046**	2.82,	<i>p</i> = .082
OTR	.217 ± .022	.170 ± .035	.091 ± .03 §	4.71,	<i>p</i> < .025
MOVT	8.1 ± .4	6.2 ± .6 **	5.3 ± 1.2	3.09,	<i>p</i> = .067
CTR	.340 ± .035	.424 ± .031	.406 ± .019	2.32,	<i>p</i> = .123
PHDR	.354 ± .031	.677 ± .056‡	.784 ± .090§	12.33,	<i>p</i> < .000
PSAR	.537 ± .045	.770 ± .053§	.859 ± .047‡	11.70,	<i>p</i> < .000
TE	31.0 ± 2.1	30.1 ± 2.0	25.0 ± 1.3 **	3.01,	<i>p</i> = .071
CE	22.6 ± 2.2	22.5 ± 2.0	21.4 ± 1.7	.12,	<i>p</i> = .886
THD	27.8 ± 3.5	20.1 ± 3.4	14.4 ± 2.1 †	4.79,	<i>p</i> < .025
HTR	.443 ± .028	.413 ± .017	.504 ± .032	3.08,	<i>p</i> = .067

Table 3. Values = mean ± SEM. Time ratios represent the amount of time (sec) on a section ÷ total time (300 sec). TE, total arm entries; OE, open arm entries; OER, open arm entry ratio; OTR, open arm time ratio; MOVT, mean time (sec) per open arm visit; CE, closed arm entries; CTR, closed arm time ratio; HTR, centre hub time ratio; THD, total head dips; PHDR, protected head dip ratio; PSAR, protected stretch attend ratio. Following oneway ANOVA, treatment group means were compared with vehicle group means. (Significant 1-tailed difference from control group: ** *p* < .025, † *p* < .01, § *p* < .005, ‡ *p* < .000 after Levene's *df* adjustment for unequal variances between groups).

revealed that NorBNI reduced the number of open entries ($F_{2,21} = 5.034, p < .025$). Post hoc comparisons revealed that the 10 nmol norBNI concentration reduced OE ($p < .01$) compared with vehicle-treated mice see (Fig. 9). NorBNI did not reduce the OER ($F_{2,21} = 2.82, NS$).

It is noteworthy that when originally tested separately, the 10 nmol concentration was

sufficient to reduce ($p < .025$) the OER (see Fig. 10). In addition, norBNI reduced the

percentage of trial time spent out in the open arms ($F_{2,21} = 4.712$, $p < .025$). This anxiogenic effect was produced by the 10 nmol ($p < .005$) concentration compared with saline controls (see Fig. 11). NorBNI did not reduce the MOVT ($F_{2,21} = 3.09$, NS). Interestingly, 1-tailed

t-tests revealed that the 1 nmol norBNI concentration was sufficient to reduce the MOV_T ($p < .025$), but most likely due to high variability the 10 nmol concentration ($p < .029$) did not significantly reduce the MOV_T (see Fig. 12). Surprisingly, the CTR was not significantly affected by either concentration of norBNI ($F_{2,21} = 2.32$, NS). The PHDR was, however, increased by this κ antagonist ($F_{2,21} = 12.328$, $p < .000$). Post hoc comparisons revealed that the 1 nmol ($p < .000$) and the 10 nmol ($p < .005$) concentrations increased the PHDR compared to mice challenged with saline (see Figure 13). Similarly, norBNI exerted an

anxiogenic effect on the PSAR ($F_{2,21} = 11.701$, $p < .000$). The 1 nmol ($p < .005$) and 10 nmol ($p < .000$) norBNI concentrations increased the PSAR compared with the saline control group (see Fig. 14). In contrast to the inhibitory effects on locomotor activity exerted by U-69,593 in the medial prefrontal cortex, norBNI did not alter the number of closed arm or total arm entries ($F_{2,21} = .122$ and 3.007 , NS) respectively. It is again worth noting that when

tested separately the 10 nmol norBNI concentration reduced TE ($p < .025$) relative to vehicle mice (see Fig. 15). In a similar fashion, norBNI reduced exploratory activity as measured

by THD ($F_{2,21} = 4.786$, $p < .025$). This apparent anxiogenic effect was exerted by the 10 nmol ($p < .01$), but not the 1 nmol drug concentration (see Fig. 16). Finally, conflict/decision (HTR) activity was not increased by norBNI ($F_{2,21} = 3.084$, NS).

U-69,593 and norBNI in the VTA. The array of cannula placements for the U-69,593 and norBNI subjects in the VTA are depicted in Plate 7. Only those subjects with correctly placed cannulae in the VTA within the rostral-caudal range defined as -2.85 mm to -3.85 mm posterior to Bregma, the dorsal-ventral range defined as -4.0 mm to -5.0 mm ventral to a flat skull surface and the medial-lateral range defined as $+0.25$ mm to $+1.0$ mm lateral to the midsagittal suture were included in the present analysis (mouse brain atlas of Franklin & Paxinos, 1997). Three mice from the 10 nmol U-69,593 group and 3 mice from the norBNI group were not included due to incorrect cannula placements anterior to the aforementioned criteria.

Plate 7. Cannula placements for the 10 nmol U-69,593 and 10 nmol norBNI subjects in the VTA. All placements were histologically verified from 40 μ m mounted brain slices stained with cresyl violet.

Plate 8. Photomicrograph of coronal section Bregma – 3.40 mm depicting a representative cannula placement in the VTA for subject # 6 of the norBNI group.

The behavioural data and t-test results pertaining to κ_1 agonist and antagonist administration in the VTA are summarized in Table 4. As was expected, there were no

U-69,593 vs. norBNI in the Ventral Tegmental Area

Behaviour	10 nmol U-69,593	10 nmol norBNI	$t_{(df)^*}$	1-tailed p
OE	11.0 $\pm$ 2.0	11.2 $\pm$ 2.4	$t_{(9.77)} = .03$,	.48 NS
OER	.437 $\pm$.068	.412 $\pm$.069	$t_{(10)} = .13$,	.40 NS
OTR	.368 $\pm$.091	.303 $\pm$.060	$t_{(8.61)} = .30$,	.28 NS
MOVT	9.8 $\pm$ 1.1	8.3 $\pm$.6	$t_{(8.02)} = .59$,	.14 NS
CTR	.318 $\pm$.055	.350 $\pm$.043	$t_{(9.42)} = .24$,	.33 NS
PHDR	.229 $\pm$.049	.321 $\pm$.071	$t_{(8.85)} = .54$,	.16 NS
PSAR	.281 $\pm$.068	.332 $\pm$.097	$t_{(8.92)} = .22$,	.34 NS
TE	24.5 $\pm$ 1.9	26.3 $\pm$ 1.9	$t_{(10)} = .34$,	.26 NS
CE	13.5 $\pm$ 1.5	15.2 $\pm$ 1.9	$t_{(9.59)} = .35$,	.25 NS
THD	28.8 $\pm$ 4.8	26.2 $\pm$ 2.7	$t_{(7.85)} = .23$,	.33 NS
HTR	.314 $\pm$.052	.346 $\pm$.040	$t_{(9.36)} = .25$,	.32 NS

Table 4. Values = mean $\pm$ SEM. Time ratios represent the amount of time (sec) on a section $\div$ total time (300 sec). OE, open arm entries; OER, open arm entry ratio; OTR, open arm time ratio; MOVT, mean open visit time (sec); CTR, closed arm time ratio; PHDR, protected head dip ratio; PSAR, protected stretch attend ratio; TE, total arm entries; CE, closed arm entries; THD, total head dips; HTR, centre hub time ratio. (df/s^* are adjusted because of unequal variances between groups).

differences in the behavioural profiles in the elevated plus maze between U-69,593 and norBNI following microinjection in the VTA. Data pertaining to drug/site comparisons are summarized in Table 5. Note that the 10 nmol U-69,593 concentration produced a significant

Drug/Site Comparisons on Elevated Plus Maze Behaviour							
	0 nmol saline		10 nmol U-69,593		10 nmol norBNI		
Behaviour	mPFC		mPFC	VTA	mPFC		VTA
OE	8.4 ± 1.2		8.4 ± 1.2	11.0 ± 2.0	3.6 ± 1.1 †		11.2 ± 2.4
OER	.276 ± .036		.370 ± .058	.437 ± .068	.147 ± .046**		.412 ± .069
OTR	.217 ± .022		.348 ± .050**	.368 ± .091	.091 ± .03 §		.303 ± .060
MOVT	8.1 ± .4		12.5 ± .4 ‡	9.8 ± 1.1	5.3 ± 1.2		8.3 ± .6
CTR	.340 ± .035		.306 ± .038	.318 ± .055	.406 ± .019		.350 ± .043
PHDR	.354 ± .031		.321 ± .049	.229 ± .049	.784 ± .090§		.321 ± .071
PSAR	.537 ± .045		.280 ± .075**	.281 ± .068**	.859 ± .047‡		.332 ± .097
TE	31.0 ± 2.1		23.5 ± 1.1 †	24.5 ± 1.9 **	25.0 ± 1.4 **		26.3 ± 1.9
CE	22.6 ± 2.2		15.1 ± 2.1 **	13.5 ± 1.5 §	21.4 ± 1.7		15.2 ± 1.9**
THD	27.8 ± 3.5		34.0 ± 3.9	28.8 ± 4.8	14.4 ± 2.1 †		26.3 ± 2.7
HTR	.443 ± .028		.337 ± .026**	.314 ± .052	.504 ± .032		.346 ± .040

Table 5. Values = mean ± SEM. Time ratios represent the amount of time (sec) on a section ÷ total time (300 sec). OE, open arm entries; OER, open arm entry ratio; OTR, open arm time ratio; MOVT, mean open visit time (sec); CTR, closed arm time ratio; PHDR, protected head dip ratio; PSAR, protected stretch attend ratio; TE, total arm entries; CE, closed arm entries; THD, total head dips; HTR, centre hub time ratio. Treatment group means were compared with the vehicle group means. (Significant difference from control group: ** $p < .025$, † $p < .01$, § $p < .005$, ¶ $p < .001$, ‡ $p < .000$ after Levene's df adjustment for unequal variances).

anxiolytic profile in the elevated plus maze following microinjection in the medial prefrontal cortex that was no longer evident following microinjection in the VTA. In contrast, an anxiogenic behavioural profile in the elevated plus maze was produced by the 10 nmol norBNI concentration in the medial prefrontal cortex that was also no longer observed from the VTA. Following Levene's adjustment for unequal variances between groups, 1-tailed t-tests indicated that although U-69,593 did not alter OE from the medial prefrontal cortex, norBNI significantly reduced OE ($p < .01$) from this cortical site (see Fig. 17). Likewise, U-69,593 did not significantly increase the OER from the mesocortex, yet norBNI

reduced the OER ($p < .025$) from this cortical site (see Fig. 18), anxiogenic effects no longer evident from the VTA relative to control mice (see Table 5). The OTR was significantly increased by U-69,593 ($p < .025$), and reduced by norBNI ($p < .005$) in the mesocortex (see

Fig. 19). As expected, both the anxiolytic and anxiogenic behavioural effects on the OTR

exerted by U-69,593 and norBNI respectively in the medial prefrontal cortex were no longer evident from the VTA (see Table 5). Interestingly, neither drug condition nor brain site

significantly influenced the CTR relative to vehicle treated controls in Experiment 2.

The protected risk assessment anxiety related measures were also differentially effected by drug and site, but in a different manner than the traditional anxiety related behavioural indices pertaining to arm related entry and time ratios at least insofar as the PSAR is concerned (see Table 5). Although the PHDR was not reduced by U-69,593 from either brain site, nor BNI significantly increased the the PHDR from the medial prefrontal cortex ($p < .005$), but again was devoid of any influence on this behavioural measure from the VTA relative to saline mice (see Fig. 20). Interestingly, the PSAR was significantly reduced by U-69,593 following microinjection in the medial prefrontal cortex ($p < .025$),

effects which were maintained ($p < .025$) from the VTA (see Fig. 21). In contrast, norBNI significantly increased the PSAR from the mesocortex ($p < .000$), effects no longer evident following microinjection in the VTA. Finally, the novel anxiety-related behavioral index (MOVT) measured in the present study was significantly increased by U-69,593 in the

medial prefrontal cortex ($p < .000$), but was not effected from the VTA (see Fig. 22).

Although norBNI reduced the MOVT ($p = .029$, NS) from the medial prefrontal cortex, it was completely devoid of influence on the MOVT from the VTA relative to vehicle treated

mice (see Table 5). Locomotor activity exhibited by CD-1 mice in the elevated plus maze was affected by both drugs in both sites, but again in a differential manner. U-69,593 clearly reduced TE from the mesocortex ($p < .01$) and the VTA ($p < .025$) compared with control mice (see Fig. 23). Notably, norBNI also reduced TE from the medial prefrontal

cortex ($p < .025$), but not from the VTA. Interestingly, although U-69,593 significantly reduced CE from the mesocortex ($p < .025$) as well as from the VTA ($p < .005$), norBNI did not reduce CE from the mesocortex, but did from the VTA ($p < .025$) relative to saline controls (see Fig. 24). Exploratory behaviour was not for the most part affected by drug condition from either brain site, with the notable exception of norBNI significantly reducing THD ($p < .01$) from the medial prefrontal cortex (see Fig. 25). Finally, U-69,593 significantly reduced the HTR from the mesocortex ($p < .025$), effects not evident from the

VTA (see Fig. 26). NorBNI did not effect the HTR relative to vehicle treated mice from either brain site.

Discussion

The behavioural results derived from Experiment 2 provide evidence for mesocortical involvement in the regulation of behavioural responsivity to an anxiety-provoking environment in CD-1 mice. Although there is a substantial literature consistent with the view that the medial prefrontal cortex is particularly sensitive to stressful or anxiogenic manipulations, most studies have not evaluated behavioural alterations following anxiogenic or anxiolytic drug administration in the mesocortex (see for example, Copland and Balfour, 1987; Abercrombie et al, 1989; Shanks et al, 1991; Zacharko and Anisman, 1991; Kaneyuki et al, 1991; Yoshioka et al, 1995). In addition, the data from Experiment 2 reveal that the κ_1 receptor agonist, U-69,593, and antagonist, norBNI, are associated with the anxiolytic or anxiogenic behavioural changes respectively among CD -1 mice in the elevated plus maze following microinjection in this cortical site. In contrast, U-69,593 was largely devoid of anxiolytic effects from the VTA, although protected risk assessment behaviours were decreased. NorBNI was devoid of anxiogenic effects from the VTA. Taken together, these data are unique in demonstrating central kappa opioid receptor involvement in anxiety-related behaviour in the elevated plus maze following challenge in the medial prefrontal cortex. Moreover, these data provide behavioural evidence describing the responsivity of the mesocortical DA/CCK system to kappa receptor challenge in the elevated plus maze.

There have been conflicting data from several laboratories regarding the use of the elevated plus maze in evaluating the anxiolytic potential of putative anxiolytic compounds (Handley and McBlane, 1993; Handley et al, 1993; McBlane and Handley, 1994; Dawson and Tricklebank, 1995; see Hogg, 1996 for review). Several different factors (e.g., maze

design and experimental protocol) likely underly such discrepancies among laboratories (Privette and Terrian, 1995; Fernandes and File, 1996). Preliminary trials were conducted in this laboratory to determine testing parameters in the elevated plus maze conducive to the production of baseline levels of open arm activity of a sufficient magnitude (i.e., 20-25% OTR, 25-30% OER) to detect either anxiolytic or anxiogenic drug influences in Experiment 2. It is noteworthy that such conditions have been reported to bias drug effects in an anxiogenic direction (File and Hitchcott, 1990; Privette and Terrian, 1995). The failure of U-69,593 or norBNI to produce floor or ceiling effects on behaviour would preclude this possibility. The fact that the observed anxiolytic and anxiogenic drug effects on behaviour in the elevated plus maze in Experiment 2 were dose-related, favours such an interpretation.

The elevated plus maze has been routinely employed by several laboratories in evaluating the relative contribution of specific brain sites to anxiety-related behavioural responding (see for example, Kostowski et al, 1989; Briley, Chopin and Moret, 1990; Treit and Pesold, 1990; Guimarães, Carobrez, De Aguiar and Graeff, 1991; Critchley, Njung'e and Handley, 1992; Pesold & Treit, 1992; Wright, Upton and Marsden, 1992; Vasar et al, 1993b; Guimarães et al, 1993; File and Gonzalez, 1996; File et al, 1996; Graeff et al, 1996). Consequently, site-specific anxiety-related behavioural changes following pharmacological manipulations in the posterior nucleus accumbens, hippocampus, central nucleus of the amygdala, nucleus of the solitary tract, septum, dorsal and median raphe nuclei, dorsal periaqueductal gray and hypothalamus among others have been reliably demonstrated in the elevated plus maze. For example, Netto and Guimarães (1996) demonstrated that bilateral microinjection of the non-selective 5-HT agonist, 5-MeODMT, in the dorsal hippocampus produced anxiolytic effects in the elevated plus maze in rats. File et al (1996) demonstrated

that 8-OH-DPAT produced anxiogenic effects from the dorsal hippocampus and anxiolytic effects from the median raphe in rats in the elevated plus maze, whereas 8-OH-DPAT was anxiolytic from the dorsal raphe and the 5-HT_{1A} antagonist, tertatolol, was anxiolytic from the ventral hippocampus in the elevated plus maze. Graeff et al (1996) also demonstrated that microinjection of 8-OH-DPAT, 5-MeODMT or the 5-HT uptake inhibitor, zimelidine, in the dorsal hippocampus reversed the deficits in open arm exploration produced 24 hrs after restraint in rats. Audi et al (1991) demonstrated that the β -NE/5-HT_{1A} antagonist, propranolol, was anxiolytic in the elevated plus maze following microinjection in the dorsal periaqueductal gray in rats. Finally, CCK-8S is anxiogenic in the elevated plus maze following microinjection in the posterior nucleus accumbens (Dauge et al, 1989; Derrien et al, 1993; Ladurelle et al, 1995) or the central nucleus of the amygdala in rats (Belcheva et al, 1994). Results from Experiment 2 not only lend support to the use of the elevated plus maze for such purposes, but also suggest that the medial prefrontal cortex can also influence behavioural/emotional responsivity to anxiety-provoking situations.

The range of traditional anxiety-related behavioural indices employed in the present study are consistent with the use of comparable measures in other laboratories. Some investigators however have apparently restricted the sensitivity of such tests by employing traditional anxiety-related indices (i.e. cumulative open or closed arm entries, open arm entry- and time-dependent ratios and total arm entries) in rats (Pellow et al, 1985) and mice (Lister, 1987). Although it is conceivable that the efficacy of the elevated plus maze in evaluating anxiety was compromised by including ethologically-derived behavioural measures, this does not seem to be the case in the present study (Rodgers and Cole, 1993a, 1993b; Dawson and Tricklebank, 1995; Hogg, 1996). For example, several investigators have demonstrated

that mice and rats engage in considerable risk assessment activity in the elevated plus maze, previously characterized as antipredator/defensive-related by Blanchard et al (1990). These measures appear to be sensitive to stressor challenge, as well as anxiogenic and anxiolytic drug administration (Cole and Rogers, 1994; Rodgers, Cole and Davies, 1994). In addition, the proportion of risk assessment behaviours expressed in protected areas of the maze (i.e., protected stretch attend postures and protected head dipping) appear to be primarily related to anxiety (Cruz, Frei and Graeff, 1994; Rodgers and Johnson, 1995; Fernandes and File, 1996). Moreover, data from several studies have revealed that protected risk assessment indices detect anxiolytic or anxiogenic drug effects in mice when traditional anxiety-related indices fail to do so (Rodgers, Cole and Tredwell, 1995; Rodgers et al, 1996). For example, Cole and Rodgers (1994) demonstrated that low-dose systemic buspirone administration produced a selective reduction in protected stretch attend postures and closed arm returns, but did not alter traditional anxiety-related behaviours. Likewise, Rodgers, Nikulina and Cole (1994) reported that although low-dose systemic sulpiride challenge selectively reduced protected head dips and protected stretch attend postures, higher doses were required to enhance open arm activity. These data provide a favourable account of the sensitivity of protected risk assessment measures to subtle changes in anxiety in mice in the elevated plus maze. The remaining behavioural indices employed in the present Experiment focused attention on otherwise overlooked behaviours, as well as the relationship of these behaviours with the spatiotemporal distribution of section preference in the apparatus (Rodgers and Johnson, 1995). For example, an animal's overall tendency to explore, a trait arguably comprising some anxiety-related components, can be reliably detected in the elevated plus maze. Rodgers and Johnson (1995) confirmed that the total number of protected plus

unprotected head dips is a sensitive index of exploratory activity in the elevated plus maze. In view of the observation that THD is decreased by anxiogenic drug administration (e.g., Cole et al, 1995). In addition, the ratio of time spent in the centre platform of the maze reliably reflects the relative degree of approach-avoidance conflict perceived by the test animal (Almeida, Garcia and De Oliveira, 1993). It is interesting that CD-1 mice in the present study performed virtually all protected risk assessment behaviours from the centre platform. Collectively, the range of behavioural indices in the present analysis not only relate to anxiety and to some degree locomotor activity, but also address behavioural dimensions which appear to be related to exploration, risk assessment and decision making (Rodgers and Johnson, 1995). In this respect, the behavioural data from Experiment 2 conceivably reflect diverse features of anxiety (i.e., approach/avoidance conflict, overt avoidance and decision making) (Cruz et al, 1994; Rodgers and Johnson, 1995).

The notion that opioids are involved in emotional responding to stressful environments is not a new one (e.g., see Stein et al, 1992). Some investigators have suggested that opioid activation may be a necessary component of anxiolysis in animal models of anxiety. For example, Ågmo et al (1995) demonstrated that systemic naloxone administration blocked the anxiolytic properties of systemic diazepam administration in the elevated plus maze in rats. It was suggested that the proconflict effects of naloxone could have been mediated through kappa receptor inhibition (Ågmo et al, 1995). The suggestion that kappa receptor ligands may influence the expression of anxiety in the elevated plus maze gained support when Privette and Terrian (1995) demonstrated that low-dose systemic administration of U-50,488H or U-69,593 produced an anxiolytic behavioural profile on some of the traditional anxiety-related measures in rats, effects which were blocked by high-

dose systemic naloxone. Specifically, 10 µg/kg U-50,488H increased OE, the OER and OTR. Likewise, the 100 µg/kg dose of U-69,593 increased OE and the OTR (Privette and Terrian, 1995). However, U-50,488H also significantly increased the HTR and TE, effects that could well be interpreted as anxiogenic and locomotor stimulating respectively, and decreased CE, suggesting locomotor suppression effects by this specific κ_1 agonist in these rats (Privette and Terrian, 1995). It is noteworthy that the 1 mg/kg dose augmented behavioural avoidance in rats in the elevated plus maze. In retrospect, it would have been interesting to see if norBNI could have blocked the anxiolytic effects produced by low-dose systemic kappa agonist administration.

Interestingly, the 10 nmol/µl concentration of U-69,593 produced the strongest anxiolytic impact on the anxiety-related measures in the elevated plus maze from the mesocortex in Experiment 2. Thus, although the 100 nmol U-69,593 concentration was for the most part devoid of anxiolytic effects relative to vehicle-treated mice suggests that the 100 nmol U-69,593 concentration likely exceeded the effective dose range capable of producing anxiolytic effects on plus maze behaviour from the mesocortex. Consistent with the anxiolytic effects produced by low-dose systemically-administered kappa agonists on open arm activity in rats (Privette and Terrian, 1995), the 10 nmol U-69,593 concentration increased the percentage of time mice spent in the open arms relative to saline treated mice. Surprisingly, this anxiolytic effect on the OTR did not arise from a reduced CTR, but rather from a reduced HTR. In addition, although neither concentration of U-69,593 influenced the number of open arm entries from the medial prefrontal cortex, both the 10 nmol and 100 nmol U-69,593 concentrations significantly increased the average duration per open arm visit. These data suggest that the MOVT may be a more sensitive anxiety index than the

frequency of open arm visits per se, since lengthened open arm visit durations would permit mice time to engage in unprotected risk assessment and exploration activity. The reduction in the HTR produced by U-69,593 from the medial prefrontal cortex would support such an interpretation. In view of the observation that U-69,593 apparently removed a significant portion of the approach-avoidance conflict effects of the apparatus, the spatio/temporal distribution of maze section preference was consequently altered in an anxiolytic direction. Whereas vehicle-treated mice preferred the centre hub > closed arms > open arms, mice from the 10 nmol U-69,593 treatment condition preferred the open arms > centre hub > closed arms. In effect, mice challenged with the 10 nmol concentration of U-69,593 were not only more decisive than control animals, but were also more inclined to explore the open arms of the elevated plus maze.

The reduction in the HTR produced by the 10 nmol U-69,593 concentration from the medial prefrontal cortex contrasts with an increase in this conflict measure produced by systemic U-50,488H administration in rats (Privette and Terrian, 1995). Interestingly, these investigators attributed the increase in the HTR among rats in the elevated plus maze to an anxiolytic effect of U-50,488H. It would appear that since these investigators did not measure the PSAR or PHDR, then the increased HTR may have been misinterpreted. In this respect, the reduced HTR among CD-1 mice from the 10 nmol U-69,593 condition was accompanied by a significant reduction in the PSAR. Together these data suggest that U-69,593 administration in the medial prefrontal cortex reduced anxiety in the open arms of the maze, reduced overt unconditioned avoidance behaviour, and facilitated decision making which decreased time spent in the centre platform of the maze.

It would appear that the anxiolytic effects produced by U-69,593 from the medial prefrontal cortex may be compromised owing to reductions in both TE and CE produced by the 10 and 100 nmol U-69,593 concentrations. Close inspection of the data, however, does not favour the interpretation that reductions in CE and TE represent reduced levels of locomotor activity. On the one hand, the reductions in TE occurred at the expense of CE (OE was not affected). On the other hand, the CTR was not reduced, suggesting that the mean duration per closed arm visit was increased. Indeed, the 100 nmol U-69,593 concentration nearly doubled the MCVT compared with saline-treated mice (data not shown). Finally, mice from either drug concentration were active in the closed arms with few if any instances of freezing postures. Accordingly, alterations in the frequency of arm entries does not appear to adequately address the issue as to whether drug-induced changes in locomotor activity can be meaningfully extracted from drug-induced changes in anxiety among laboratory animals. The suggestion that drug-induced reductions in CE not only reflects inhibition of locomotor activity but also confounds the interpretability of drug-induced anxiolysis in the elevated plus maze (Rodgers and Johnson, 1995) is further brought into question as some laboratories interpret reductions in CE as an anxiolytic effect of drug treatment (e.g., see Privette and Terrian, 1995). The TE and CE data from Experiment 2 are in accordance with data derived from other studies however, where quite often investigators have demonstrated that systemic administration of various putative anxiolytic compounds which produce anxiolytic effects on open arm time and entry ratios, also reduce TE and/or CE. For example, Rodgers et al (1996) observed that systemic administration of the D_2/D_3 receptor agonist, 7-OH-DPAT, at the highest dose tested increased the OER and decreased the CTR and PHDR, but at the same time reduced CE and TE in DBA/2 mice. In a similar fashion, systemic administration

of the D₂ antagonist, sulpiride, in male DBA/2 mice produced a significant anxiolytic profile in traditional as well as novel measures that was also accompanied by a significant reduction in CE (Rodgers, Nikolina and Cole, 1994). Furthermore, systemic buspirone administration was anxiolytic in DBA/2 mice on both arm and risk assessment behaviours in the elevated plus maze, but also reduced CE (Cole and Rodgers, 1994), findings reproduced by systemic 8-OH-DPAT (Rodgers et al, 1992), the selective 5-HT_{1A} agonist, flesinoxan (Rodgers, Cole and Davies, 1994) as well as systemic yohimbine administration (Cole et al, 1995) in male DBA/2 mice. In view of these data, the reductions in CE evident among CD-1 mice in Experiment 2 is consistent with the induction of an anxiolytic effect of U-69,593 from the medial prefrontal cortex.

In contrast to the anxiolytic behavioural effects in the elevated plus maze produced by U-69,593 from the medial prefrontal cortex, norBNI produced an extensive dose-dependent anxiogenic behavioural profile from this cortical site in Experiment 2, with the 10 nmol norBNI concentration producing the most anxiogenic behavioural effects. In view of the observation that the 10 nmol concentration of norBNI was capable of producing a conspicuous anxiogenic effect among the first 3 subjects tested, a lower concentration of norBNI (i.e., 1 nmol/ μ l) was employed to determine if the lower concentration was sufficient to influence at least some of the anxiety-related behaviours selected. This did not occur as the 1 nmol norBNI concentration was not sufficient to significantly reduce the open arm activity- or time-dependent ratios. It is noteworthy however that the dose response curves depicting OE, the OER and OTR illustrate the anxiogenic trend produced by the 1 nmol norBNI concentration on these traditional anxiety-related indices. Clearly, the 10 nmol norBNI concentration was sufficient to reduce OE, the OER and OTR from the mesocortex

relative to vehicle-treated mice. Notably, no floor effect was evident in any of the three corresponding dose-response curves, suggesting that concentrations in excess of 10 nmol could conceivably have produced further reductions in open arm activity. In addition, the 1 nmol norBNI concentration was sufficient to reduce the MOVt. Taken together, the four indices related to open arm activity were altered by norBNI from the mesocortex.

It is interesting that norBNI provoked a dose-dependent anxiogenic effect on the PSAR and PHDR relative to saline controls. These data provide further evidence to support the notion that protected risk assessment measures are indeed sensitive anxiety-related indices in the elevated plus maze (Rodgers & Johnson, 1995). The 1 nmol and 10 nmol norBNI concentrations dose-dependently increased the PHDR and the PSAR. It would appear that norBNI produced a greater magnitude of anxiogenic effects on traditional anxiety-related and protected risk assessment measures from the medial prefrontal cortex than the U-69,593-induced anxiolytic effects on these behavioural indices. This observation holds for the exploration index as well. In this respect, the 10 nmol norBNI concentration significantly reduced THD from the medial prefrontal cortex relative to control mice.

In keeping with the position that reductions in CE do not necessarily reflect reductions in locomotor activity but perhaps indicate anxiolytic drug activity, one would expect an anxiogenic drug to increase CE. Neither the 1 nmol nor the 10 nmol norBNI concentration altered CE from the mesocortex in the present study. Total entries were reduced by the 10 nmol norBNI concentration however, but unlike the reductions in TE elicited by U-69,593 from the mesocortex which were entirely at the expense of CE, the reductions in TE were at the expense of OE. Taken together, these data indicate that, on the one hand, if an anxiolytic drug reduces TE in the elevated plus maze while concomitantly

reducing CE but not OE, then the reductions in TE (despite no change in OE) could be interpreted as an anxiolytic drug effect. On the other hand, if an anxiogenic drug reduces TE while concomitantly reducing OE but not CE, then the reductions in TE (despite no change in CE) could be interpreted as an anxiogenic drug effect. Clearly, reductions in TE could be interpreted either way, depending on the changes, if any, in CE and OE. Thus reductions in TE produced by the 10 nmol norBNI concentration from the medial prefrontal cortex in the present study could be interpreted as an anxiogenic drug effect rather than a decrease in locomotor activity per se. Finally, the 10 nmol norBNI concentration marginally increased the HTR from the mesocortex where mice from this group spent slightly over 50% of the time in the centre platform of the maze. This is clearly reflected by the high levels of protected risk assessment activity evident among the 10 nmol norBNI-treated mice. Accordingly, the spatio-temporal distribution of maze section preference observed among mice in the 10 nmol norBNI condition shows that these mice preferred the centre hub > closed arms > open arms. These data suggest that norBNI-treated mice were not only more indecisive than saline-treated mice, but also avoided the open arms of the maze at least 90 % of the time in the present study.

There does not appear to be any available data from which to compare the behavioural effects of intracerebral anxiogenic drug administration in the elevated plus maze in mice, particularly data involving kappa receptor antagonists. In view of the anxiolytic and anxiogenic profiles produced by U-69,593 and norBNI respectively from the medial prefrontal cortex, kappa receptors within this cortical site likely mediated these drug-induced behavioural changes in the elevated plus maze. In order to define the site-specific influence

of kappa receptors in the medial prefrontal cortex. norBNI and U-69,593 were microinjected in the VTA in independent groups of CD-1 mice.

Clearly, there were no differences in behavioural responding evident between groups following microinjection of either 10 nmol U-69, 593 or 10 nmol norBNI in the VTA. These findings were not surprising based on available autoradiographic, immunohistochemical and in situ hybridization data demonstrating few if any κ_1 receptors or binding sites in the VTA (Mansour et al, 1988; Mansour et al, 1995). There is no doubt however that stressor activation of the mesocorticolimbic DA/CCK system is partly mediated by endogenous opioid release in the VTA (Sesack and Pickel, 1992; Kalivas, 1993). Furthermore, it has been demonstrated that stressor-induced opioid release in the VTA may enhance DA release in the medial prefrontal cortex in rats (Kalivas and Arnold, 1987; Kalivas, 1993). However, there are accumulating data demonstrating that kappa agonists do not exert an appreciable influence on locomotor activity following microinjection in the VTA (e.g., see Kalivas et al, 1985; Jenck et al, 1987a, 1987b). It would appear, at first glance, that the anxiety-related data from the VTA in Experiment 2 are for the most part consistent with this interpretation.

Virtually all of the anxiogenic effects produced by the 10 nmol norBNI concentration on plus maze behaviour from the medial prefrontal cortex in CD-1 mice were not evident following microinjection in the VTA. In addition, it is noteworthy that there were no significant differences evident in the anxiety-related behavioural indices between the norBNI VTA group and the vehicle control group from the medial prefrontal cortex. These data add impetus to the view that norBNI exerted site-specific anxiogenic influences on behavioural responding in the elevated plus maze from the medial prefrontal cortex. The U-69,593 data from the VTA in the present study are not as straight forward to interpret however. Thus,

although U-69,593 no longer displayed significant anxiolytic influences on open arm activity from the VTA, 10 nmol U-69,593 reduced the PSAR from the VTA relative to control mice.

In view of the aforementioned degree of sensitivity accorded protected risk assessment behavioural indices, the apparent anxiolytic effects produced by the selective κ_1 agonist, U-69,593, on the PSAR from the VTA cannot be discounted. Perhaps a higher U-69,593 concentration in the VTA could have produced anxiolytic effects on the PHDR, as well as on some of the traditional open arm indices. In addition, U-69,593 reduced both TE and CE from the VTA as it did from the mesocortex. Consistent with the notion that reductions in TE which occur at the expense of CE indicate anxiolytic drug effects, U-69,593 produced a small, but detectable, anxiolytic influence from the VTA. Taken together, these data suggest that the low density of κ_1 receptors in the VTA compared to the moderate to high density in the medial prefrontal cortex may not be the distinguishing factor explaining the differences in anxiolytic profiles following microinjection of U-69,593 in the mesocortex versus the VTA. Rather, κ_1 receptor sensitivity may also address the observed behavioural differences. Thus, whereas the 100 nmol U-69,593 concentration appeared to exceed the dose range capable of exerting anxiolytic effects from the medial prefrontal cortex, it would have been of interest to determine if the 100 nmol concentration of the κ_1 agonist would exert an anxiolytic influence from the VTA. Clearly, the lack of anxiolytic effects produced by the κ_1 agonist, U-69,593, on traditional behavioural indices from the VTA in the present study does not rule out the possible involvement of the A10 area in the production or reduction of anxiety-related behaviour. Taken together, κ_1 receptor sensitivity as well as drug dose may partially explain the small anxiolytic effects produced by U-69,593 from the VTA. Interestingly, norBNI did not reduce TE from the VTA as it did in the

mesocortex, but significantly reduced CE from the VTA, an effect it did not exert from the medial prefrontal cortex. With these observations in mind, there were distinct differences between the behavioural profiles produced by norBNI from the VTA relative to the medial prefrontal cortex. In this respect, it could be suggested that the anxiogenic effects produced by norBNI from the medial prefrontal cortex in the elevated plus maze were site-specific.

It is interesting that U-69,593 and norBNI produced opposite effects on anxiety-related behaviour in CD-1 mice from the medial prefrontal cortex. In addition, U-69,593 produced a small anxiolytic effect from the VTA. These findings are in accordance with suggestions from several investigators that the mesocortical DA/CCK system is involved in anxiety-related behavioural processes. In fact, some investigators have argued that stressor-induced activation of mesocortical DA metabolism is related to anxiety or fear in laboratory animals (e.g., see Biggio et al, 1990 for review). Such a hypothesis suggests that enhanced DA metabolic activity in the medial prefrontal cortex is associated with the behavioural expression of anxiety. This interpretation stems from neurochemical data demonstrating that systemic β -carboline administration elevates DOPAC concentrations in the medial prefrontal cortex in rats (Tam & Roth, 1990), as does acute low-intensity footshock, exposure to cues associated with footshock (Deutch, Tam & Roth, 1985) or exposure to a novel environment in mice (Tassin et al, 1980). These data, coupled with the observation that prior BDZ treatment prevents the enhanced DOPAC levels in the mesocortex following footshock (Tam & Roth, 1990), gave impetus to the suggestion that enhanced DA metabolism in the medial prefrontal cortex and the VTA (Deutch et al, 1985) contributes to the behavioural expression of anxiety. Kaneyuki et al (1991) also demonstrated that DOPAC concentrations in the medial prefrontal cortex were selectively enhanced in rats placed in proximity to the sight,

sound and smell of other rats receiving footshock, effects also attenuated by BDZ pretreatment. These data, although convincing, could only infer an anxiogenic role for DA metabolic activation in the medial prefrontal cortex.

Recent *in vivo* data suggest that DOPAC levels derived from postmortem tissue analysis (primarily from intracellular sources) is a much less sensitive index of DA activity than direct estimation of DA release *in vivo* following either pharmacological or stressor manipulations (Abercrombie et al, 1989; Imperato et al, 1990). In accordance with this view, it will be recalled that whereas various stressors such as mild restraint, mild footshock or exposure to a novel environment selectively enhance mesocortical DOPAC levels in rats as derived from postmortem tissue samples (Tassin, 1980; Deutch et al, 1985), these same stressor parameters actually can enhance DA release in the mesocortex, nucleus accumbens, or the striatum depending on the nature and severity of the stressor (Imperato et al, 1990; Imperato et al, 1993). Moreover, although systemic diazepam administration decreases basal spontaneous DA release in the mesocortex, it does not decrease the enhanced DA release in the mesocortex and nucleus accumbens induced by restraint in rats (Imperato et al, 1990). Taken together, these data do not support the notion that enhanced mesocortical DA metabolic activity translates into the behavioural expression of anxiety. Accordingly, enhanced DA release in the medial prefrontal cortex may be a necessary neurochemical response to counteract otherwise anxiety-provoking neurochemical cascades evoked by anxiogenic situations. Moreover, the failure of systemic BDZ administration to attenuate stressor-induced mesocortical DA release in rats suggests an anxiolytic role for DA in this cortical site. Otherwise, if stressor-enhanced DA release in the medial prefrontal cortex were to produce anxiogenic effects, one would expect that systemic BDZ administration would

inhibit such release. In this respect, the anxiolytic effects produced by U-69,593 from the medial prefrontal cortex in Experiment 2 would support this notion. Indeed, had microinfused U-69,593 reduced DA release in the medial prefrontal cortex, then some behavioural changes likely would have been observed among CD-1 mice in the elevated plus maze including enhanced avoidance behaviour for example. Perhaps the observation that low U-69,593 concentrations reduced some of the threatening aspects of the apparatus following microinjection in the medial prefrontal cortex in CD-1 mice would favour such a conclusion.

Some investigators have suggested that, since cortical CCK_B receptor activation is associated with anxiety in laboratory animals, then a reduction of CCK release should reduce anxiety-related behaviour (Raiteri et al, 1996). In view of the propensity of CCK to enhance GABA release in the mesocortex (Perez de la Mora et al, 1993; Ferraro et al, 1996), as well as the propensity of GABA to reduce DA release in rats in the mesocortex (Santiago et al, 1993), suggests that U-69,593 may produce anxiolytic effects on plus maze behaviour through inhibition of CCK release in the medial prefrontal cortex. The observation that κ_1 receptors are found on mesocortical DA/CCK terminals would support this notion (Mansour et al, 1988, Mansour, Fox, Akil and Watson, 1995). In contrast, κ_1 receptor activation in the VTA may have decreased somatodendritic DA release and conceivably enhanced mesocortical DA neurotransmission. Taken together, it would be naive to suggest that κ_1 receptors reduced anxiety in CD-1 mice through interactions with only DA and/or CCK in the mesocortical system. By their very nature however, one major function of opioids such as dynorphin is to inhibit transmitter release through the activation of κ_1 receptors (Donzanti et al, 1992; Devine et al, 1993). Accordingly, it is conceivable that U-69,593 could inhibit 5-HT release from the medial prefrontal cortex for example. This notion deserves mention

as it will be recalled that 5-HT enhances CCK release in the prefrontal cortex in freely moving rats (Raiteri et al, 1993). In this respect, it is noteworthy that a) the exposure of rats to the same environment in which footshock was delivered produces conditioned freezing behaviour concomitant with enhanced 5-HT release in the medial prefrontal cortex and b) the conditioned freezing and enhanced 5-HT release in these rats were prevented by the 5-HT₃ antagonist, tropisetron (Yoshioka et al, 1995). In addition, Vasar et al (1993a) demonstrated that the 5-HT₃ antagonist, ondansetron, blocked the anxiety-related behaviour provoked by the CCK agonist, caerulein, in the elevated plus maze in rats. It would be interesting to examine whether U-69,593 could reduce 5-HT release from the medial prefrontal cortex in rats in the elevated plus maze. Such data would add impetus to the notion that kappa receptors may also be expressed on 5-HT terminals in this cortical site.

In view of the aforementioned possible neurochemical explanations for the drug-induced effects observed among CD-1 in Experiment 2, the question remains as to what terminal location(s) and/or neuromechanism(s) were involved in the U-69,593- and norBNI-induced anxiolytic and anxiogenic behavioural effects respectively in the medial prefrontal cortex. The fact that the behavioural effects produced by these κ receptor ligands were salient from the mesocortex attests to the unlikelyhood that these behavioural effects could have been produced by drug diffusion, for example, into the striatum. Microdialysis studies have shown that κ receptor agonists dose-dependently decrease DA release and metabolism in the striatum concomitant with significant reductions in spontaneous locomotor activity (e.g., see Di Chiara and Imperato, 1988). It would appear that the 1 μ l volume of each drug injection in the medial prefrontal cortex was unlikely to have induced such prominent effects on anxiety from the striatum in CD-1 mice. In addition, the mechanisms involved in

the small anxiolytic effects produced by U-69,593 from the VTA are also not known. It is conceivable that, in addition to expression in DA dendritic sites, κ_1 receptors may also be expressed in afferent neuronal terminals or interneuronal cell bodies in the A10 area. Furthermore, U-69,593 may have exerted some effects on dendritic or afferent terminal CCK release in the VTA in CD-1 mice. Taken together, future studies should evaluate the effects of other opioids such as μ or σ agonists or antagonists, as well as the recently developed D_4 receptor agonists or antagonists for example, on anxiety-related behaviour in the elevated plus maze from the VTA.

In the present study, progress has been made toward the understanding of the role kappa opioids play in an animal's emotional responding to threatening environments. It is noteworthy that the elevated plus maze does not inflict physical pain or constraints (i.e., restraint) on test animals, therefore arguably addresses unconditioned psychological facets of anxiety inherent in mice and rats. Certainly, kappa opioid receptors in the medial prefrontal cortex cannot be solely responsible for the behavioural effects observed among CD-1 mice in Experiment 2. Rather, kappa opioid activation in the mesocortical system may be a necessary (or facilitative) neurochemical component of anxiolysis. In conclusion, data from the present study indicate that the medial prefrontal cortex may not only just serve as a perceptual filter coupled to some level of spatio/temporal mnemonic function (Mogensen & Holm, 1994), but also may very well anatomically regulate an animal's short- and long-term visceromotor/behavioural responsivity in anxiety-provoking environments such as the elevated plus maze. Finally, the role of the VTA, or perhaps a sub area of the VTA (e.g., see Deutch, Tam and Roth, 1985), in the neurocircuitry underlying anxiety appears somewhat enigmatic. In any event, the A10 area should be considered along with the medial

prefrontal cortex when evaluating the contributions of the mesocortical DA/CCK system to the behavioural expression of anxiety.

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Appendix A

1. 1-tailed t-test of **Variable Housing** on Open Entries (OE)

Levene's Test for Equality of Variances: $F = 5.458$, $p = .035$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Group Housed	8	3.5000	.598	Equal	1.74	14	.05
Single Housed	8	6.5000	1.615	Unequal	1.74	8.88	.058

2. 1-tailed t-test of **Variable Housing** on Open Arm Entry Ratio (OER)

Levene's Test for Equality of Variances: $F = 3.675$, $p = .076$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Group Housed	8	.2108	.035	Equal	1.46	14	.08
Single Housed	8	.3124	.060	Unequal	1.46	11.24	.09

3. 1-tailed t-test of **Variable Housing** on Open Time Ratio (OTR)

Levene's Test for Equality of Variances: $F = 7.657$, $p = .015$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Group Housed	8	.1131	.019	Equal	1.95	14	.036
Single Housed	8	.2143	.048	Unequal	1.95	9.12	.04

4. 1-tailed t-test of **Variable Housing** on Mean Open Visit Time (MOVT)

Levene's Test for Equality of Variances: $F = .046$, $p = .834$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Group Housed	8	8.6925	1.452	Equal	1.01	14	.16
Single Housed	8	10.5963	1.199	Unequal	1.01	13.52	.16

5. 1-tailed t-test of **Variable Housing** on Closed Arm Time Ratio (CTR)

Levene's Test for Equality of Variances: $F = .256$, $p = .621$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Group Housed	8	.3175	.024	Equal	.09	14	.47
Single Housed	8	.3141	.030	Unequal	.09	13.36	.47

6. 1-tailed t-test of **Variable Housing** on Protected Head Dip Ratio (PHDR)

Levene's Test for Equality of Variances: $F = 1.937$, $p = .186$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Group Housed	8	.6380	.063	Equal	1.38	14	.09
Single Housed	8	.4706	.104	Unequal	1.38	11.50	.09

7. 1-tailed t-test of **Variable Housing** on Protected Stretch Attend Ratio (PSAR)

Levene's Test for Equality of Variances: $F = 1.263$, $p = .280$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Group Housed	8	.7366	.047	Equal	.62	14	.27
Single Housed	8	.6903	.058	Unequal	.62	13.40	.27

8. 1-tailed t-test of **Variable Housing** on Total Arm Entries (TE)

Levene's Test for Equality of Variances: $F = 4.324$, $p = .056$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Group Housed	8	16.6250	.778	Equal	1.71	14	.054
Single Housed	8	20.1250	1.894	Unequal	1.71	9.30	.06

9. 1-tailed t-test of **Variable Housing** on Total Arm Entries (TE)

Levene's Test for Equality of Variances: $F = 7.313$, $p = .017$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Group Housed	8	13.1250	.811	Equal	.30	14	.38
Single Housed	8	13.6250	1.463	Unequal	.30	10.93	.39

10. 1-tailed t-test of **Variable Housing** on Total Head Dips (THD)

Levene's Test for Equality of Variances: $F = 2.216$, $p = .159$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Group Housed	8	16.5000	2.732	Equal	1.18	14	.13
Single Housed	8	25.1250	6.747	Unequal	1.18	9.24	.13

11. 1-tailed t-test of **Variable Housing** on Centre Hub Time Ratio (HTR)

Levene's Test for Equality of Variances: $F = 17.380$, $p = .001$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Group Housed	8	.5694	.016	Equal	2.71	14	.017
Single Housed	8	.4717	.032	Unequal	2.71	10.40	.021

Appendix B

1. **ANOVA Summary Table**
U-69,593 in the medial prefrontal cortex on Open Arm Entries (OE)

Source	<i>df</i>	S. Squares	M. Square	<i>F</i>	<i>p</i>
Between Groups	2	1.3333	.6667	.0393	.9616
Within Groups	21	356.6250	16.9821		
Total	23	357.9583			

2. **ANOVA Summary Table**
U-69,593 in the medial prefrontal cortex on Open Arm Entry Ratio (OER)

Source	<i>df</i>	S. Squares	M. Square	<i>F</i>	<i>p</i>
Between Groups	2	.0362	.0181	.8337	.4483
Within Groups	21	.4562	.0217		
Total	23	.4924			

3. **ANOVA Summary Table**
U-69,593 in the medial prefrontal cortex on Open Arm Time Ratio (OTR)

Source	<i>df</i>	S. Squares	M. Square	<i>F</i>	<i>p</i>
Between Groups	2	.0694	.0347	1.9687	.1646
Within Groups	21	.3703	.0176		
Total	23	.4398			

4. **ANOVA Summary Table**
U-69,593 in the medial prefrontal cortex on Mean Open Arm Visit Time (MOVT)

Source	<i>df</i>	S. Squares	M. Square	<i>F</i>	<i>p</i>
Between Groups	2	86.2943	43.1472	12.6116	.0003
Within Groups	21	71.8455	3.4212		
Total	23	158.1399			

5. **ANOVA Summary Table**
U-69,593 in the medial prefrontal cortex on Closed Arm Time Ratio (CTR)

Source	<i>df</i>	S. Squares	M. Square	<i>F</i>	<i>p</i>
Between Groups	2	.0222	.0111	.8516	.4409
Within Groups	21	.2742	.0131		
Total	23	.2964			

6. **ANOVA Summary Table**
U-69,593 in the medial prefrontal cortex on Protected Head Dip Ratio (PHDR)

Source	<i>df</i>	S. Squares	M. Square	<i>F</i>	<i>p</i>
Between Groups	2	.0277	.0139	.9678	.3962
Within Groups	21	.3006	.0143		
Total	23	.3283			

7. **ANOVA Summary Table**
U-69,593 in the medial prefrontal cortex on Prot. Stretch Attend Ratio (PSAR)

Source	<i>df</i>	S. Squares	M. Squares	<i>F</i>	<i>p</i>
Between Groups	2	.2669	.1335	4.0969	.0315
Within Groups	21	.6841	.0326		
Total	23	.9511			

8. **ANOVA Summary Table**
U-69,593 in the medial prefrontal cortex on Total Arm Entries (TE)

Source	<i>df</i>	S. Squares	M. Square	<i>F</i>	<i>p</i>
Between Groups	2	277.0833	138.5417	5.7398	.0103
Within Groups	21	506.8750	24.1369		
Total	23	783.9583			

9. **ANOVA Summary Table**
 U-69,593 in the medial prefrontal cortex on Closed Arm Entries (CE)

Source	<i>df</i>	S. Squares	M. Square	<i>F</i>	<i>p</i>
Between Groups	2	261.7500	130.8750	4.6406	.0214
Within Groups	21	592.2500	28.2024		
Total	23	854.0000			

10. **ANOVA Summary Table**
 U-69,593 in the medial prefrontal cortex on Total Head Dips (THD)

Source	<i>df</i>	S. Squares	M. Square	<i>F</i>	<i>p</i>
Between Groups	2	193.0000	96.5000	.5514	.5842
Within Groups	21	3675.0000	175.0000		
Total	23	3868.0000			

11. **ANOVA Summary Table**
 U-69,593 in the medial prefrontal cortex on Centre Hub Time Ratio (HTR)

Source	<i>df</i>	S. Squares	M. Square	<i>F</i>	<i>p</i>
Between Groups	2	.0596	.0298	5.5529	.0116
Within Groups	21	.1128	.0054		
Total	23	.1724			

Appendix C

1. 1-tailed t-test of U-69,593 (10 nmol) in mPFC on Open Entries (OE)

Levene's Test for Equality of Variances: $F = .026$, $p = .873$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0nmol mPFC	8	8.3750	1.149	Equal	.00	14	1.000
10nmol mPFC	8	8.3750	1.209	Unequal	.00	13.96	1.000

2. 1-tailed t-test of U-69,593 (10 nmol) in mPFC on Open Entry Ratio (OER)

Levene's Test for Equality of Variances: $F = .615$, $p = .446$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0nmol mPFC	8	.2759	.036	Equal	1.37	14	.95
10nmol mPFC	8	.3696	.058	Unequal	1.37	11.73	.10

3. 1-tailed t-test of U-69,593 (10 nmol) in mPFC on Open Time Ratio (OTR)

Levene's Test for Equality of Variances: $F = 2.650$, $p = .126$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0nmol mPFC	8	.2165	.022	Equal	2.44	14	.015
10nmol mPFC	8	.3483	.050	Unequal	2.44	9.57	.018

4. 1-tailed t-test of U-69,593 (10 nmol) in mPFC on Mean Open Visit Time (MOVT)

Levene's Test for Equality of Variances: $F = .141$, $p = .713$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0nmol mPFC	8	8.1025	.395	Equal	8.03	14	.000
10nmol mPFC	8	12.4862	.377	Unequal	8.03	13.97	.000

5. 1-tailed t-test of U-69,593 (10 nmol) in mPFC on Closed Time Ratio (CTR)

Levene's Test for Equality of Variances: $F = .478$, $p = .501$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	.3404	.035	Equal	.67	14	.26
10 nmol mPFC	8	.3058	.038	Unequal	.67	13.85	.26

6. 1-tailed t-test of U-69,593 (10 nmol) in mPFC on Prot. Head Dip Ratio (PHDR)

Levene's Test for Equality of Variances: $F = 1.279$, $p = .277$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	.3536	.031	Equal	.57	14	.29
10 nmol mPFC	8	.3210	.049	Unequal	.57	11.79	.29

7. 1-tailed t-test of U-69,593 (10 nmol) in mPFC on Prot. Stretch Attend Ratio (PSAR)

Levene's Test for Equality of Variances: $F = 1.069$, $p = .319$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	.5373	.045	Equal	2.94	14	.011
10 nmol mPFC	8	.2795	.075	Unequal	2.94	11.44	.013

8. 1-tailed t-test of U-69,593 (10 nmol) in mPFC on Total Arm Entries (TE)

Levene's Test for Equality of Variances: $F = 4.892$, $p = .044$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	31.0000	2.130	Equal	3.12	14	.008
10 nmol mPFC	8	23.5000	1.118	Unequal	3.12	10.59	.010

9. 1-tailed t-test of U-69,593 (10 nmol) in mPFC on Closed Entries (CE)

Levene's Test for Equality of Variances: $F = .452$, $p = .512$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0nmol mPFC	8	22.6250	2.179	Equal	2.50	14	.013
10nmol mPFC	8	15.1250	2.057	Unequal	2.50	13.95	.013

10. 1-tailed t-test of U-69,593 (10 nmol) in mPFC on Total Head Dips (THD)

Levene's Test for Equality of Variances: $F = .254$, $p = .622$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0nmol mPFC	8	27.7500	3.519	Equal	1.19	14	.13
10nmol mPFC	8	34.0000	3.882	Unequal	1.19	13.87	.13

11. 1-tailed t-test of U-69,593 (10 nmol) in mPFC on Centre Hub Time Ratio (HTR)

Levene's Test for Equality of Variances: $F = .021$, $p = .887$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0nmol mPFC	8	.443	.028	Equal	2.77	14	.015
10nmol mPFC	8	.337	.026	Unequal	2.77	13.95	.015

Appendix C (cont'd)

12. 1-tailed t-test of U-69,593 (100 nmol) in mPFC on Open Arm Entries (OE)

Levene's Test for Equality of Variances: $F = 1.412$, $p = .254$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	8.375	1.149	Equal	.23	14	.413
100 nmol mPFC	8	7.875	1.894	Unequal	.23	11.54	.413

13. 1-tailed t-test of U-69,593 (100 nmol) in mPFC on Open Entry Ratio (OER)

Levene's Test for Equality of Variances: $F = 2.462$, $p = .139$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	.2759	.036	Equal	.48	14	.32
100 nmol mPFC	8	.3086	.059	Unequal	.48	11.69	.32

14. 1-tailed t-test of U-69,593 (100 nmol) in mPFC on Open Time Ratio (OTR)

Levene's Test for Equality of Variances: $F = 8.176$, $p = .013$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	.2165	.022	Equal	1.02	14	.16
100 nmol mPFC	8	.2821	.061	Unequal	1.02	8.74	.17

15. 1-tailed t-test of U-69,593 (100 nmol) in mPFC on Mean Open Visit Time (MOVT)

Levene's Test for Equality of Variances: $F = 3.518$, $p = .082$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	8.1025	.395	Equal	3.30	14	.005
100 nmol mPFC	8	11.6238	.993	Unequal	3.30	9.16	.009

16. 1-tailed t-test of U-69,593 (100 nmol) in mPFC on Closed Time Ratio (CTR)

Levene's Test for Equality of Variances: $F = 2.419$, $p = .142$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	.3404	.035	Equal	.68	14	.25
100 nmol mPFC	8	.3802	.047	Unequal	.68	12.81	.25

17. 1-tailed t-test of U-69,593 (100 nmol) in mPFC on Prot. Head Dip Ratio (PHDR)

Levene's Test for Equality of Variances: $F = 1.220$, $p = .288$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	.3536	.031	Equal	1.51	14	.08
100 nmol mPFC	8	.2710	.046	Unequal	1.51	12.23	.08

18. 1-tailed t-test of U-69,593 (100 nmol) in mPFC on Prot. Stretch Att. Ratio (PSAR)

Levene's Test for Equality of Variances: $F = 1.773$, $p = .204$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	.5373	.045	Equal	1.41	14	.09
100 nmol mPFC	8	.4234	.067	Unequal	1.41	12.23	.09

19. 1-tailed t-test of U-69,593 (100 nmol) in mPFC on Total Arm Entries (TE)

Levene's Test for Equality of Variances: $F = .987$, $p = .337$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	31.0000	2.130	Equal	2.46	14	.014
100 nmol mPFC	8	24.1250	1.807	Unequal	2.46	13.64	.014

20. 1-tailed t-test of U-69,593 (100 nmol) in mPFC on Closed Arm Entries (CE)

Levene's Test for Equality of Variances: $F = 6.045$, $p = .028$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	22.6250	2.179	Equal	2.53	14	.012
100 nmol mPFC	8	16.2500	1.264	Unequal	2.53	11.23	.014

21. 1-tailed t-test of U-69,593 (100 nmol) in mPFC on Total Head Dips (THD)

Levene's Test for Equality of Variances: $F = 3.322$, $p = .090$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	27.7500	3.519	Equal	.07	14	.47
100 nmol mPFC	8	28.2500	6.178	Unequal	.07	11.11	.46

22. 1-tailed t-test of U-69,593 (100 nmol) in mPFC on Centre Hub Time Ratio (HTR)

Levene's Test for Equality of Variances: $F = .042$, $p = .840$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	.4431	.028	Equal	2.90	14	.012
100 nmol mPFC	8	.3374	.023	Unequal	2.90	13.61	.012

Appendix D

1. ANOVA Summary Table
norBNI in the med. Prefrontal Cortex on Open Arm Entries (OE)

Source	<i>df</i>	S. Squares	M. Square	<i>F</i>	<i>p</i>
Between Groups	2	104.3333	52.1667	5.0339	.0164
Within Groups	21	217.6250	10.3631		
Total	23	321.9583			

2. ANOVA Summary Table
norBNI in the med. Prefrontal Cortex on Open Arm Entry Ratio (OER)

Source	<i>df</i>	S. Squares	M. Square	<i>F</i>	<i>p</i>
Between Groups	2	.0768	.0384	2.8220	.0821
Within Groups	21	.2856	.0136		
Total	23	.3623			

3. ANOVA Summary Table
norBNI in the med. Prefrontal Cortex on Open Arm Time Ratio (OTR)

Source	<i>df</i>	S. Squares	M. Square	<i>F</i>	<i>p</i>
Between Groups	2	.0648	.0324	4.7123	.0204
Within Groups	21	.1444	.0069		
Total	23	.2092			

4. ANOVA Summary Table
norBNI in the med. Prefrontal Cortex on Mean Open Visit Time (MOVt)

Source	<i>df</i>	S. Squares	M. Square	<i>F</i>	<i>p</i>
Between Groups	2	33.8021	16.9010	3.0898	.0666
Within Groups	21	114.8704	5.4700		
Total	23	148.6725			

5. ANOVA Summary Table
norBNI in the med. Prefrontal Cortex on Closed Arm Time Ratio (CTR)

Source	<i>df</i>	S.Squares	M. Square	<i>F</i>	<i>p</i>
Between Groups	2	.0308	.0154	2.3195	.1230
Within Groups	21	.1396	.0066		
Total	23	.1704			

6. ANOVA Summary Table
norBNI in the med. Prefrontal Cortex on Protected Head Dip Ratio (PHDR)

Source	<i>df</i>	S. Squares	M. Square	<i>F</i>	<i>p</i>
Between Groups	2	.8018	.4009	12.3276	.0003
Within Groups	21	.6829	.0325		
Total	23	1.4847			

7. ANOVA Summary Table
norBNI in the med. Prefrontal Cortex on Prot. Stretch Attend Ratio (PSAR)

Source	<i>df</i>	S. Squares	M. Square	<i>F</i>	<i>p</i>
Between Groups	2	.4406	.2203	11.7010	.0004
Within Groups	21	.3954	.0188		
Total	23	.8361			

8. ANOVA Summary Table
norBNI in the med. Prefrontal Cortex on Total Arm Entries (TE)

Source	<i>df</i>	S. Squares	M. Square	<i>F</i>	<i>p</i>
Between Groups	2	168.0833	84.0417	3.0072	.0710
Within Groups	21	586.8750	27.9464		
Total	23	754.9583			

9. **ANOVA Summary Table**
norBNI in the med. Prefrontal Cortex on Closed Arm Entries (CE)

Source	<i>df</i>	S. Squares	M. Square	<i>F</i>	<i>p</i>
Between Groups	2	7.5833	3.7917	.1222	.8856
Within Groups	21	651.7500	31.0357		
Total	23	659.3333			

10. **ANOVA Summary Table**
norBNI in the med. Prefrontal Cortex on Total Head Dips (THD)

Source	<i>df</i>	S. Squares	M. Square	<i>F</i>	<i>p</i>
Between Groups	2	720.2500	360.1250	4.7857	.0194
Within Groups	21	1580.2500	75.2500		
Total	23	2300.5000			

11. **ANOVA Summary Table**
norBNI in the med. Prefrontal Cortex on Centre Hub Time Ratio (HTR)

Source	<i>df</i>	S. Squares	M. Square	<i>F</i>	<i>p</i>
Between Groups	2	.0342	.0171	3.0838	.0669
Within Groups	21	.1164	.0055		
Total	23	.1506			

Appendix E

1. 1-tailed t-test of **norBNI** (1 nmol) in the mPFC on Open Arm Entries (OE)

Levene's Test for Equality of Variances: $F = .115$, $p = .740$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	8.3750	1.149	Equal	.46	14	.33
1 nmol mPFC	8	7.6250	1.179	Unequal	.46	13.99	.33

2. 1-tailed t-test of **norBNI** (1 nmol) in the mPFC on Open Arm Entry Ratio (OER)

Levene's Test for Equality of Variances: $F = .223$, $p = .644$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	.2759	.036	Equal	.39	14	.35
1 nmol mPFC	8	.2547	.041	Unequal	.39	13.80	.35

3. 1-tailed t-test of **norBNI** (1 nmol) in the mPFC on Open Arm Time Ratio (OTR)

Levene's Test for Equality of Variances: $F = 2.610$, $p = .129$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	.2165	.022	Equal	1.14	14	.14
1 nmol mPFC	8	.1699	.035	Unequal	1.14	11.69	.14

4. 1-tailed t-test of **norBNI** (1 nmol) in the mPFC on Mean Open Visit Time (MOVT)

Levene's Test for Equality of Variances: $F = 1.254$, $p = .282$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	8.1025	.395	Equal	2.67	14	.018
1 nmol mPFC	8	6.2113	.589	Unequal	2.67	12.24	.020

5. 1-tailed t-test of **norBNI** (1 nmol) in the mPFC on Closed Arm Time Ratio (CTR)

Levene's Test for Equality of Variances: $F = .003$, $p = .959$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	.3404	.035	Equal	1.81	14	.05
1 nmol mPFC	8	.4239	.031	Unequal	1.81	13.81	.05

6. 1-tailed t-test of **norBNI** (1 nmol) in the mPFC on Prot. Head Dip Ratio (PHDR)

Levene's Test for Equality of Variances: $F = 3.046$, $p = .103$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	.3536	.031	Equal	5.03	14	.000
1 nmol mPFC	8	.6766	.056	Unequal	5.03	10.77	.000

7. 1-tailed t-test of **norBNI** (1 nmol) in the mPFC on Prot. Stretch Att. Ratio (PSAR)

Levene's Test for Equality of Variances: $F = .370$, $p = .553$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	.5373	.045	Equal	3.34	14	.005
1 nmol mPFC	8	.7701	.053	Unequal	3.34	13.63	.005

8. 1-tailed t-test of **norBNI** (1 nmol) in the mPFC on Total Arm Entries (TE)

Levene's Test for Equality of Variances: $F = .010$, $p = .922$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	31.0000	2.130	Equal	.30	14	.38
1 nmol mPFC	8	30.1250	2.004	Unequal	.30	13.95	.38

9. 1-tailed t-test of **norBNI** (1 nmol) in the mPFC on Closed Arm Entries (CE)

Levene's Test for Equality of Variances: $F = .551$, $p = .470$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	22.6250	2.179	Equal	.04	14	.48
1 nmol mPFC	8	22.5000	1.982	Unequal	.04	13.88	.48

10. 1-tailed t-test of **norBNI** (1 nmol) in the mPFC on Total Head Dips (THD)

Levene's Test for Equality of Variances: $F = .133$, $p = .721$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	27.7500	3.519	Equal	1.57	14	.07
1 nmol mPFC	8	20.1250	3.362	Unequal	1.57	13.97	.07

11. 1-tailed t-test of **norBNI** (1 nmol) in the mPFC on Centre Hub Time Ratio (HTR)

Levene's Test for Equality of Variances: $F = .833$, $p = .377$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	.4431	.028	Equal	.92	14	.18
1 nmol mPFC	8	.4130	.017	Unequal	.92	11.66	.19

Appendix E (cont'd)

12. 1-tailed t-test of **norBNI** (10 nmol) in the mPFC on Open Arm Entries (OE)

Levene's Test for Equality of Variances: $F = .000$, $p = 1.000$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	8.3750	1.149	Equal	3.01	14	.009
10 nmol mPFC	8	3.6250	1.085	Unequal	3.01	13.95	.009

13. 1-tailed t-test of **norBNI** (10 nmol) in the mPFC on Open Arm Entry Ratio (OER)

Levene's Test for Equality of Variances: $F = 1.001$, $p = .334$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	.2759	.036	Equal	2.21	14	.022
10 nmol mPFC	8	.1468	.046	Unequal	2.21	13.31	.025

14. 1-tailed t-test of **norBNI** (10 nmol) in the mPFC on Open Arm Time Ratio (OTR)

Levene's Test for Equality of Variances: $F = 2.308$, $p = .151$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	.2165	.022	Equal	3.41	14	.004
10 nmol mPFC	8	.0906	.030	Unequal	3.41	12.71	.005

15. 1-tailed t-test of **norBNI** (10 nmol) in the mPFC on Mean Open Visit Time (MOVT)

Levene's Test for Equality of Variances: $F = 6.300$, $p = .025$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	8.1025	.395	Equal	2.19	14	.023
10 nmol mPFC	8	5.2450	1.244	Unequal	2.19	8.40	.029

16. 1-tailed t-test of **norBNI** (10 nmol) in the mPFC on Closed Arm Time Ratio (CTR)

Levene's Test for Equality of Variances: $F = 2.527$, $p = .134$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	.3404	.035	Equal	1.66	14	.06
10 nmol mPFC	8	.4056	.019	Unequal	1.66	10.86	.06

17. 1-tailed t-test of **norBNI** (10 nmol) in the mPFC on Prot. Head Dip Ratio (PHDR)

Levene's Test for Equality of Variances: $F = 4.110$, $p = .062$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	.3536	.031	Equal	4.53	14	.000
10 nmol mPFC	8	.7836	.090	Unequal	4.53	8.59	.002

18. 1-tailed t-test of **norBNI** (10 nmol) in the mPFC on Prot. Stretch Att. Ratio (PSAR)

Levene's Test for Equality of Variances: $F = .156$, $p = .698$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	.5373	.045	Equal	4.93	14	.000
10 nmol mPFC	8	.8585	.047	Unequal	4.93	13.97	.000

19. 1-tailed t-test of **norBNI** (10 nmol) in the mPFC on Total Arm Entries (TE)

Levene's Test for Equality of Variances: $F = 2.213$, $p = .159$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	31.0000	2.130	Equal	2.36	14	.016
10 nmol mPFC	8	25.0000	1.389	Unequal	2.36	12.04	.017

20. 1-tailed t-test of **norBNI** (10 nmol) in the mPFC on Closed Arm Entries (CE)

Levene's Test for Equality of Variances: $F = 1.950$, $p = .184$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	22.6250	2.179	Equal	.45	14	.33
10 nmol mPFC	8	21.3750	1.721	Unequal	.45	13.29	.33

21. 1-tailed t-test of **norBNI** (10 nmol) in the mPFC on Total Head Dips (THD)

Levene's Test for Equality of Variances: $F = .478$, $p = .501$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	27.7500	3.519	Equal	3.25	14	.006
10 nmol mPFC	8	14.3750	2.129	Unequal	3.25	11.52	.007

22. 1-tailed t-test of **norBNI** (10 nmol) in the mPFC on Centre Hub Time Ratio (CTR)

Levene's Test for Equality of Variances: $F = .085$, $p = .774$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
0 nmol mPFC	8	.4431	.028	Equal	1.44	14	.09
10 nmol mPFC	8	.5038	.032	Unequal	1.44	13.77	.09

Appendix F

1. 1-tailed t-test of U-69 vs. BNI in the VTA (10 nmol) on Open Arm Entries (OE)

Levene's Test for Equality of Variances: $F = .270$, $p = .614$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
norBNI	6	11.1667	2.372	Equal	.05	10	.48
U-69,593	6	11.0000	2.033	Unequal	.05	9.77	.48

2. 1-tailed t-test of U-69 vs. BNI in the VTA (10 nmol) on Open Entry Ratio (OER)

Levene's Test for Equality of Variances: $F = .018$, $p = .896$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
norBNI	6	.4120	.069	Equal	.26	10	.40
U-69,593	6	.4372	.068	Unequal	.26	10.00	.40

3. 1-tailed t-test of U-69 vs. BNI in the VTA (10 nmol) on Open Time Ratio (OTR)

Levene's Test for Equality of Variances: $F = 1.909$, $p = .197$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
norBNI	6	.3033	.060	Equal	.59	10	.28
U-69,593	6	.3682	.091	Unequal	.59	8.61	.28

4. 1-tailed t-test of U-69 vs. BNI in the VTA (10 nmol) on M. Open Visit Time (MOVt)

Levene's Test for Equality of Variances: $F = .639$, $p = .443$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
norBNI	6	8.3283	.619	Equal	1.18	10	.14
U-69,593	6	9.7883	1.068	Unequal	1.18	8.02	.14

5. 1-tailed t-test of **U-69** vs. **BNI** in the VTA (10 nmol) on Closed Time Ratio (CTR)

Levene's Test for Equality of Variances: $F = .015$, $p = .905$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
norBNI	6	.3503	.043	Equal Unequal	.47	10	.33
U-69,593	6	.3175	.055		.47	9.42	.33

6. 1-tailed t-test of **U-69** vs. **BNI** in the VTA (10 nmol) on Prot. Head Dip Ratio (PHDR)

Levene's Test for Equality of Variances: $F = .199$, $p = .665$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
norBNI	6	.3212	.071	Equal Unequal	1.07	10	.15
U-69,593	6	.2287	.049		1.07	8.85	.16

7. 1-tailed t-test of **U-69** vs. **BNI** in the VTA (10 nmol) on Pr. Stretch Att. Ratio (PSAR)

Levene's Test for Equality of Variances: $F = 1.675$, $p = .225$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
norBNI	6	.3322	.097	Equal Unequal	.43	10	.34
U-69,593	6	.2813	.068		.43	8.92	.34

8. 1-tailed t-test of **U-69** vs. **BNI** in the VTA (10 nmol) on Total Arm Entries (TE)

Levene's Test for Equality of Variances: $F = .022$, $p = .886$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
norBNI	6	26.3333	1.944	Equal Unequal	.67	10	.26
U-69,593	6	24.5000	1.910		.67	10	.26

9. 1-tailed t-test of **U-69** vs. **BNI** in the VTA (10 nmol) on Closed arm Entries (CE)

Levene's Test for Equality of Variances: $F = .006$, $p = .942$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
norBNI	6	15.1667	1.851	Equal	.70	10	.25
U-69.593	6	13.5000	1.500	Unequal	.70	9.59	.25

10. 1-tailed t-test of **U-69** vs. **BNI** in the VTA (10 nmol) on Total Head Dips (THD)

Levene's Test for Equality of Variances: $F = .544$, $p = .478$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
norBNI	6	26.3333	2.692	Equal	.45	10	.33
U-69.593	6	28.8333	4.813	Unequal	.45	7.85	.33

11. 1-tailed t-test of **U-69** vs. **BNI** in the VTA (10 nmol) on Hub Time Ratio (HTR)

Levene's Test for Equality of Variances: $F = .604$, $p = .455$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
norBNI	6	.3463	.040	Equal	.50	10	.32
U-69.593	6	.3138	.052	Unequal	.50	9.36	.32

Appendix G

1. 1-tailed t-test of U-69 in VTA vs. **saline** in mPFC on Open Arm Entries (OE)

Levene's Test for Equality of Variances: $F = .719$, $p = .413$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Sal mPFC	8	8.3750	1.149	Equal	1.20	12	.13
U-69 VTA	6	11.0000	2.033	Unequal	1.12	8.11	.15

2. 1-tailed t-test of U-69 in VTA vs. **saline** in mPFC on Open Entry Ratio (OER)

Levene's Test for Equality of Variances: $F = 1.146$, $p = .305$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Sal mPFC	8	.2759	.036	Equal	2.25	12	.022
U-69 VTA	6	.4372	.068	Unequal	2.10	7.82	.035

3. 1-tailed t-test of U-69 in VTA vs. **saline** in mPFC on Open Time Ratio (OTR)

Levene's Test for Equality of Variances: $F = 14.184$, $p = .003$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Sal mPFC	8	.2165	.022	Equal	1.85	12	.045
U-69 VTA	6	.3682	.091	Unequal	1.62	5.56	.08

4. 1-tailed t-test of U-69 in VTA vs. **saline** in mPFC on Mean Open Visit Time (MOVT)

Levene's Test for Equality of Variances: $F = 1.44$, $p = .253$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Sal mPFC	8	8.1025	.395	Equal	1.65	12	.062
U-69 VTA	6	9.7883	1.068	Unequal	1.48	6.38	.093

5. 1-tailed t-test of **U-69** in VTA vs. **saline** in mPFC on Closed Time ratio (CTR)

Levene's Test for Equality of Variances: $F = .120$, $p = .735$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Sal mPFC	8	.3404	.035	Equal	.35	12	.36
U-69 VTA	6	.3175	.055	Unequal	.35	8.69	.37

6. 1-tailed t-test of **U-69** in VTA vs. **saline** in mPFC on Prot. Head Dip Ratio (PHDR)

Levene's Test for Equality of Variances: $F = 1.318$, $p = .273$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Sal mPFC	8	.3536	.031	Equal	2.28	12	.021
U-69 VTA	6	.2287	.049	Unequal	2.17	8.72	.039

7. 1-tailed t-test of **U-69** in VTA vs. **saline** in mPFC on Prot. Stretch Att. Ratio (PSAR)

Levene's Test for Equality of Variances: $F = .671$, $p = .429$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Sal mPFC	8	.5373	.045	Equal	3.28	12	.007
U-69 VTA	6	.2813	.068	Unequal	3.15	9.12	.012

8. 1-tailed t-test of **U-69** in VTA vs. **saline** in mPFC on Total Arm Entries (TE)

Levene's Test for Equality of Variances: $F = .743$, $p = .405$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Sal mPFC	8	31.0000	2.130	Equal	2.19	12	.025
U-69 VTA	6	24.5000	1.910	Unequal	2.27	11.96	.021

9. 1-tailed t-test of **U-69** in VTA vs. **saline** in mPFC on Closed Arm Entries (CE)

Levene's Test for Equality of Variances: $F = 4.810$, $p = .049$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Sal mPFC	8	22.6250	2.179	Equal	3.21	12	.008
U-69 VTA	6	13.5000	1.500	Unequal	3.45	11.57	.005

10. 1-tailed t-test of **U-69** in VTA vs. **saline** in mPFC on Total Head Dips (THD)

Levene's Test for Equality of Variances: $F = .052$, $p = .824$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Sal mPFC	8	27.7500	3.519	Equal	.19	12	.42
U-69 VTA	6	28.8333	4.813	Unequal	.18	9.78	.43

11. 1-tailed t-test of **U-69** in VTA vs. **saline** in mPFC on Centre Hub Time Ratio (HTR)

Levene's Test for Equality of Variances: $F = 2.199$, $p = .164$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Sal mPFC	8	.4431	.028	Equal	2.35	12	.018
U-69 VTA	6	.3138	.052	Unequal	2.19	7.81	.030

For 1-tailed *t*-test tables showing comparisons between 10 nmol **U-69,593** in mPFC and **saline** in

mPFC see **Appendix B**.

Appendix H

1. 1-tailed t-test of **BNI** in VTA vs. **saline** in mPFC on Open Arm Entries (OE)

Levene's Test for Equality of Variances: $F = 2.431$, $p = .145$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Sal mPFC	8	8.3750	1.149	Equal	1.15	12	.14
BNI VTA	6	11.1667	2.372	Unequal	1.06	7.33	.16

2. 1-tailed t-test of **BNI** in VTA vs. **saline** in mPFC on Open Entry Ratio (OER)

Levene's Test for Equality of Variances: $F = 1.716$, $p = .215$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Sal mPFC	8	.2759	.036	Equal	1.88	12	.042
BNI VTA	6	.4120	.069	Unequal	1.75	7.74	.06

3. 1-tailed t-test of **BNI** in VTA vs. **saline** in mPFC on Open Time ratio (OTR)

Levene's Test for Equality of Variances: $F = 6.221$, $p = .028$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Sal mPFC	8	.2165	.022	Equal	1.53	12	.076
BNI VTA	6	.3033	.060	Unequal	1.37	6.32	.109

4. 1-tailed t-test of **BNI** in VTA vs. **saline** in mPFC on M. Open Visit Time (MOVT)

Levene's Test for Equality of Variances: $F = .099$, $p = .758$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Sal mPFC	8	8.1025	.395	Equal	.32	12	.376
BNI VTA	6	8.3283	.619	Unequal	.31	8.85	.383

5. 1-tailed t-test of **BNI** in VTA vs. **saline** in mPFC on Closed Time Ratio (CTR)

Levene's Test for Equality of Variances: $F = .084$, $p = .777$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Sal mPFC	8	.3404	.035	Equal	.18	12	.43
BNI VTA	6	.3503	.043	Unequal	.18	10.41	.43

6. 1-tailed t-test of **BNI** in VTA vs. **saline** in mPFC on Prot. Head Dip Ratio (PHDR)

Levene's Test for Equality of Variances: $F = 1.545$, $p = .238$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Sal mPFC	8	.3536	.031	Equal	.46	12	.326
BNI VTA	6	.3212	.071	Unequal	.42	6.84	.344

7. 1-tailed t-test of **BNI** in VTA vs. **saline** in mPFC on Prot. Stretch Att. Ratio (PSAR)

Levene's Test for Equality of Variances: $F = 4.937$, $p = .046$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Sal mPFC	8	.5373	.045	Equal	2.09	12	.028
BNI VTA	6	.3322	.097	Unequal	1.91	7.14	.048

8. 1-tailed t-test of **BNI** in VTA vs. **saline** in mPFC on Total Arm Entries (TE)

Levene's Test for Equality of Variances: $F = .577$, $p = .462$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Sal mPFC	8	31.0000	2.130	Equal	1.56	12	.072
BNI VTA	6	26.3333	1.944	Unequal	1.62	11.93	.066

9. 1-tailed t-test of **BNI** in VTA vs. **saline** in mPFC on Closed Arm Entries (CE)

Levene's Test for Equality of Variances: $F = 2.906$, $p = .114$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Sal mPFC	8	22.6250	2.179	Equal	2.49	12	.014
BNI VTA	6	15.1667	1.851	Unequal	2.61	12	.012

10. 1-tailed t-test of **BNI** in VTA vs. **saline** in mPFC on Total Head Dips (THD)

Levene's Test for Equality of Variances: $F = .340$, $p = .570$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Sal mPFC	8	27.7500	3.519	Equal	.30	12	.38
BNI VTA	6	26.3333	2.692	Unequal	.32	11.89	.37

11. 1-tailed t-test of **BNI** in VTA vs. **saline** in mPFC on Hub Time Ratio (HTR)

Levene's Test for Equality of Variances: $F = .474$, $p = .504$

Variable	# of Cases	Mean	SEM	Variances	t-value	df	1-Tail Sig
Sal mPFC	8	.4431	.028	Equal	2.06	12	.03
BNI VTA	6	.3463	.040	Unequal	1.99	9.46	.038

For 1-tailed *t*-test tables showing comparisons between 10 nmol **norBNI** in mPFC and **saline** in mPFC see **Appendix E**.

IMAGE EVALUATION TEST TARGET (QA-3)

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