THE EFFECTS OF INTRAVENOUS CHOLECYSTOKININ ON
NUCLEUS ACCUMBENS NEUROCHEMISTRY AND BEHAVIOUR IN
AWAKE RATS

by

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A thesis submitted in conformity with the requirements for the degree of

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Cholecystokinin (CCK) has marked anxiogenic or panicogenic effects when acutely administered to rodents, monkeys, and human panic disorder patients, via the activation of brain CCK<sub>B</sub> receptors. In parallel with its anxiogenic properties, CCK is also colocalized with dopamine (DA) in mesolimbic neurons which project to the nucleus accumbens (NAcc), where CCK interacts with DA to modulate reward-related behaviours.

In the present study, the neurochemical effects of i.v. CCK4 (a selective CCK<sub>B</sub> agonist) in the NAcc were evaluated using <i>in vivo</i> microdialysis in awake male Wistar rats. The behavioural effects of i.v. CCK4 were also tested using measures of horizontal activity and acoustic startle.

It was found that i.v. CCK4 potentiated NAcc DA:DOPAC levels approximately 20 minutes following the infusion. CCK4 also produced an immediate decrease in activity and an immediate potentiation of startle amplitudes. Implications of these results for the neurochemical basis of certain motivational processes are considered.
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Introduction

Cholecystokinin (CCK) is a peptide found throughout the central nervous system (CNS) that appears to function as a neurotransmitter. Repeated experiments in humans and animals suggest that CCK possesses powerful anxiogenic properties when administered either peripherally or into specific brain regions. Given the widespread distribution of CCK throughout the brain, the possibility exists that CCK is involved with the endogenous modulation of neuronal activity in a manner consistent with fear or anxiety in several brain regions. As such, research designed to investigate the physiological effects of CCK may lead to an enhanced understanding of the brain mechanisms that mediate these motivational processes.

CCK, in parallel with its anxiogenic properties, is also functionally present within a large proportion of mesolimbic dopamine (DA) cells projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NAcc). The importance of the mesolimbic system in the mediation of motivational processes involving reinforcement and reward is well-documented. The complex behavioural and neurochemical interactions between CCK and DA in this region, combined with the potent anxiogenic effects of systemic CCK, highlight the NAcc as one important candidate region for investigation relating to the elucidation of the motivationally relevant neurochemical cascade of events following intravenous (i.v.) CCK administration.

Neurophysiological Properties of CCK

CCK is a neuropeptide that exists in several forms within the CNS. The predominant form is the sulfated octapeptide (CCK8S) (Beinfeld, Meyer, Eskay, Jensen, & Brownstein, 1981), although it may also exist endogenously in an unsulfated form (CCK8US) and perhaps in a tetrapeptide form (CCK4). CCK is synthesized de novo (Golterman, Stengaard-Pedersen,
Rehfeld, & Christensen, 1981), is released via a calcium-dependent mechanism (Emson, Lee, & Rehfeld, 1980), and is active at, at least two distinct subreceptors: CCK\textsubscript{A} and CCK\textsubscript{B} (Moran, Robinson, Goldrich, & McHugh, 1986; Honda, Wada, Battey, & Wank, 1993). The CCK\textsubscript{A} (alimentary) receptor is found predominantly in the periphery but also in concentrated locations within the brain, including the NAcc (Abercrombie, Keefe, DiFrischia, & Zigmond, 1989), and is endogenously stimulated primarily by CCK\textsubscript{8S} (Barret, Steffey, & Wolfram, 1989). The CCK\textsubscript{B} (brain) receptor is found in abundance throughout the brain (Gaudreau, St.-Pierre, Pert, & Quinon, 1985) and is stimulated by CCK\textsubscript{8S}/US and CCK\textsubscript{4} (Lotti & Chang, 1989). In view of this evidence, it has been suggested that CCK functions as a neurotransmitter or neuromodulator (Vanderhaeghen, Deschepper, Lostra, Vierendeels, and Schoenen, 1982).

CCK is found colocalized with several neurotransmitters including mesolimbic, mesocortical and nigrostriatal DA (Hökfelt, Rehfeld, Skirboll, Ivemark, Goldstein, & Markey, 1980; Hökfelt, Skirboll, Rehfeld, Goldstein, Markey, & Dann, 1980; Seroogy & Fallon, 1989), and also GABA in the amygdala (McDonald & Pearson, 1989) and nonpyramidal cells of the hippocampus (Hp) (Seroogy, Dangaran, Lim, Haycock, & Fallon, 1989). CCK may also project from the prefrontal cortex (PFC) to the rostral NAcc--independent of DA (Fallon & Seroogy, 1985; Morino, Mascagni, McDonald, & Hökfelt, 1994; Seroogy & Fallon, 1989; Zaborsky, Alheid, Beinfeld, Eiden, Helmen, & Palkovitz, 1985; for review Crawley, 1991) although recent immunocytochemical results (ICC) challenge this possibility (Lanca, A.J., de Cabo, C., Arifuzzaman, A.I., & Vaccarino, F.J., submitted). Interestingly, Crawley (1985) reports that CCK is the most abundant peptide in the nervous system.
CCK and Anxiety

Recently, a series of studies in humans have produced evidence that CCK may be involved with panic disorder. CCK4, when i.v. bolus injected (i.e. in less than 5 sec) at µg doses, reliably produces panic attacks in panic disorder patients (Bradwejn, Koszycki, Annable, Couëtoux du Tertre, Reines, & Karkanias, 1992; Bradwejn, Koszycki, & Meterissian, 1990; Bradwejn, Koszycki, & Shiriqui, 1991; for review Bradwejn, Koszycki, Couëtoux du Tertre, Bourin, Palmour, & Ervin, 1992) and at higher doses in a proportion of healthy volunteers (Bradwejn, Koszycki, & Bourin, 1991; de Montigny, 1989). Similarly panic attacks are induced by the selective CCK₄ agonist, pentagastrin (Abelson & Nesse, 1990) and CCK-induced panic attacks in panic patients have been blocked by pretreatment with a CCK₄ antagonist, L-365,260, orally administered at a dose of 50 mg (Bradwejn, Koszycki, Couëtoux du Tertre, van Megan et al., 1994). Interestingly, endogenous levels of CCK₈ are indicated to be present at significantly lower levels in panic disorder patients relative to healthy volunteers, as evidenced by lymphocyte levels (Brambilla, Bellodi, Perna, Garberi, Panerai, & Sacerdote, 1993) and cerebrospinal fluid (CSF) levels (Lydiard, Ballenger, Laraia, Fossey, & Beinfeld, 1992). This finding raises the possibility that CCK receptors are more numerous or supersensitive in panic disorder patients.

CCK-induced anxiety has also been found in animals studies. The tetrapeptide induced panic symptoms in apes, and this effect was attenuated by pretreatment with a CCK₄ antagonist, LY262691 (Palmour, Ervin, Bradwejn, & Howbert, 1991). In rodents Singh, Lewis, Field, Hughes, & Woodruff (1991) report that CCK₄ receptor blockade (CI-988), and not CCK₆ receptor blockade (devazepide), produces anxiolytic effects in the rat elevated plus maze, rat social interaction test, rat conflict test, and mouse light/dark box, without producing sedation. This drug also dose-dependently antagonized anxiety-symptoms induced by centrally administered
pentagastrin. Incidentally, peripherally administered pentagastrin was found to be inactive in this study (see also Singh, Field et al., 1991). Harro and Vasar (1991b) report that CCK$_B$ antagonists, but not CCK$_A$ antagonists attenuate the anxiogenic effects of CCK4 (50 $\mu$g/kg) in some exploratory tests, not including the elevated plus-maze.

Endogenous CCK$_B$ receptor activation has been correlated with anxiogenesis by studies inducing behavioural anxiety without pharmacological manipulations. For instance, Pavlasevic, Bednar, Qureshi, and Södersten (1991) tested whether rats exposed to the smell of a predator would show changes in neurotransmitter levels in various brain regions using HPLC analyses of brain tissue homogenates. They found increased levels of CCK4 in the frontal cortex, dorsal striatum, NAcc, central nucleus of the amygdala, olfactory bulb and nucleus of the solitary tract. CCK8 was increased only in the NAcc. DA was elevated in the frontal cortex and glutamate (GLU) was elevated in the NAcc. Josselyn, Frankland, Petrisano, Bush, Yeomans, and Vaccarino (1995) found that L-365,260 attenuates fear-potentiated acoustic startle amplitudes without affecting baseline startle levels. Thus, under certain conditions CCK$_B$ antagonists have anxiolytic properties indicating a role for CCK in the endogenous mediation of anxiety-related neuronal processes (Costall et al., 1991; Hughes et al., 1990). Similarly, anxiogenic drugs that target other neurotransmitter systems have also been documented to increase brain CCK levels. In a recent review Raiteri, Paudice, and Vallebuona (1993) report that they have found serotonin (5-HT) 5-HT$_3$ receptor agonists to increase CCK levels in the NAcc and in the frontal cortex while simultaneously producing freezing behaviour in rats. Similarly, they report that the anxiolytic 5-HT$_3$ receptor antagonist, ondansetron (GR 38032F), inhibits CCK release in the frontal cortex. Pratt and Brett (1995) also report that FG 7142, an anxiogenic benzodiazepine receptor inverse agonist, induces gene expression of CCK in both the BLA and in the CA3 region of the Hp.
Exogenous local CNS microinjections of CCK ligands have been found to potentiate measures of anxiety in rodents. Baseline acoustic startle reflex magnitudes are also potentiated by the exogenous micro-application of CCK into the pontine reticular nucleus (Fendt, Koch, Kungel, & Schnitzler, 1995), and also by pentagastrin or CCK4 microinjections into the basolateral nucleus of the amygdala (BLA; Frankland, Josselyn, Bradwejn, Vaccarino & Yeomans, 1997; Vaccarino, Arifuzzaman et al., 1997).

Based on the above body of evidence, combined with the known importance of the amygdala in fear-related processes (Davis, 1992), it is hypothesized that CCK subtypes receptor activation at the amygdala serves as an endogenous neurochemical trigger for downstream fear-related neurotransmission. Therefore, acute systemic CCK4 administration is hypothesized to produce symptoms of anxiety in rodents.

Mesolimbic DA-CCK Interactions

As previously noted, CCK is colocalized with DA in a large proportion of mesolimbic (A10) projection neurons. This finding has given impetus to a parallel line of research that has focused on the functional role of CCK-DA interactions in the psychological processes of reward and reinforcement. Thus, the details of these complex interactions (Vaccarino, 1994) will be presently considered. Given the involvement of CCK neurotransmission in fear processes, it may be theoretically interesting to consider the anxiogenic properties of CCK subtypes receptor activation in concert with the direct or indirect effects of CCK on the mesolimbic reward system (Vaccarino, 1995).

The capacity for psychostimulant drugs such as amphetamine (AMPH) and cocaine to produce behavioural activation has been associated with their ability to pharmacologically enhance
the endogenous brain mechanisms that mediate reward. Brain reward circuits have been hypothesized to mediate stimulant-induced locomotor activation (Swerdlow, Vaccarino, Amalric, & Koob, 1986; Vaccarino, Amalric, Swerdlow, & Koob, 1986; Wise & Bozarth, 1987) and sucrose feeding (Carr & White, 1986; Colle & Wise, 1988; Evans & Vaccarino, 1986; 1987; 1990; Sills, Baird, & Vaccarino, 1993; Sills & Vaccarino, 1991). Activation of reward circuitry in the presence of stimuli can induce a preference for those stimuli. Similarly, emitted behaviours that are paired with reward system activation are reinforced, that is, the likelihood of the animal repeating the behaviour is increased (Vaccarino, Schiff, & Glickman, 1989). Finally, psychostimulant self-administration is thought to result from the direct pharmacological stimulation of neurophysiological reward systems (Collins, Weeks, Cooper, Good, & Russell, 1984; De Wit & Wise, 1977; Koob, Le, & Creese, 1987; Koob, Vaccarino, Amalric, & Bloom, 1987).

The neurophysiological substrates which mediate the rewarding and reinforcing properties of psychostimulants appear to depend on their ability to enhance mesolimbic DAergic neurotransmission. Both microinjections of DA receptor antagonists into the NAcc, and 6-hydroxydopamine-induced lesions of mesolimbic DA cells, attenuate psychostimulant-induced hyperlocomotion (Kelley, Sevoir, & Iversen, 1975; Pijnenburgh, Honig, van Rossum, 1975). DA receptor stimulation directly within the NAcc increases locomotor activity (Pijnenburgh & van Rossum, 1973), and systemic psychostimulants at doses that produce locomotor activation preferentially increase DA release in the NAcc (Sharp, Zetterström, Ljungberg, & Ungerstedt, 1987). Additionally, animals will self-administer DA agonists directly into the NAcc (Hoebel, Monaco, Hernandez, Aulisi, Stanley, & Lenard, 1983; Monaco, Hernandez, & Hoebel, 1981); furthermore, intra-NAcc 6-hydroxydopamine (6-OHDA) lesions and microinjections of DA
antagonists into the NAcc have been shown to attenuate the behavioural effects of systemically self-administered psychostimulants (Koob, Vaccarino et al., 1987; Lyness, Friedle, & Moore, 1979). Taken together these converging sources of evidence suggest that DA neurotransmission at the NAcc is a fundamental neurochemical substrate for reward (for reviews see Beninger, 1983; Koob, Vaccarino et al., 1987; LeMoal & Simon, 1991; Phillips, Pfaus, & Blaha, 1991; Vaccarino, Schiff, & Glickman, 1989; White, 1989; and Wise, 1987).

CCK is colocalized with DA in at least 40% of mesolimbic DA neurons (Vanderhaeghen, Signeau, & Gepts, 1975). However, within the NAcc there appear to be a rostral-caudal dissociation with respect to CCK innervation (Hökfelt, Rehfeld et al., 1980; Hökfelt, Skirboll et al., 1980; Hökfelt, Millhorn et al., 1987). In the caudal NAcc, CCK innervation is primarily from A10 DA cells, but in the rostral NAcc, there are reports of CCK innervation that does not originate at the VTA, and that may be independent of DA. These CCK fibres are thought by some to project from the medial PFC (Studler, Reibaud, Herve, Blanc, & Tassin, 1986; for review Crawley, 1991), or the substantia nigra (Lanca et al., submitted). DA inhibits adenylate cyclase (AC) activity in NAcc cells, and CCK8S has been shown to decrease AC activity in the caudal NAcc and, in contrast, to increase AC activity in the rostral NAcc (Studler et al., 1986).

the caudal NAcc, and decreases DA neurotransmission via CCKB receptors in the rostral NAcc (Vaccarino, 1994).

CCK affects reward-dependent behaviours in a manner consistent with its DA modulating effects. CCK potentiates and inhibits AMPH-induced locomotor activation in the caudal and rostral NAcc, respectively (Vaccarino & Rankin, 1989). CCKA receptor blockade results in the attenuation of rewarding brain stimulation (BSR), and CCKB receptor blockade induces a slight potentiation of BSR (Vaccarino & Vaccarino, 1989). Rostral NAcc microinjections of pentagastrin dose-dependently increase response rates for i.v. AMPH self-administration (Bush, DeSousa, & Vaccarino, 1996). Systemic L-365,260 potentiates AMPH-induced increases in responding for a conditioned reinforcer (Josselyn & Vaccarino, 1995). Individual differences in sucrose feeding (Sills, Baird, & Vaccarino, 1993; Sills & Vaccarino, 1991), AMPH-induced exploratory behavior (Higgins, Sills, Tomkins, Sellars, & Vaccarino, 1994), and the acquisition and expression of AMPH self-administration (DeSousa, Bush, & Vaccarino, 1996) are thought to depend on differences in DAergic tone. It is possible that CCKB receptors contribute to these DA-dependent differences (Higgins et al., 1994; Vaccarino, 1994; Vaccarino, Bush et al., 1997) since individual differences in reward responsivity appear to inversely correlate with anxiety levels. Specifically, high sugar feeders display less anxiety-related behaviour (DeSousa, Wunderlich, de Cabo, & Vaccarino, accepted) than do rodents displaying relatively lower levels of reward-related behaviour.

Anxiogenic manipulations have been found to affect NAcc DA. In fact, the systemic administration of both beta-CCE and FG 7142 increased in vivo DA release by approximately 50% in the NAcc (McCullough & Salamone, 1992) and also in the PFC (Bradberry, Lory & Roth, 1991). The stressful/anxiogenic properties of CCK challenges might be expected to have a similar
effect on mesolimbic DA. Indeed, microinjections of CCK8S (but not CCK8US or CCK4) into the VTA have been found to increase DA overflow in the NAcc and the BLA (Hamilton & Freedman, 1995), and the mesocortical and mesolimbic DA systems are clearly responsive to certain stressful manipulations (Claustre, Rivy, Dennis, & Scatton, 1986; Coco, Kuhn, Ely, & Kilts, 1989; Deutch & Roth, 1990; Herman, Guillonneau, Dantzer, Scatton, Semerdjian-Rouquier, & LeMoal, 1982; for review Bertolucci-D’Angio, Serrano, & Scatton, 1990).

It is important to note that the DA-potentiating anxiogenic manipulation in the above experiment was not accompanied by an increase in locomotor activity (McCullough & Salamone, 1992). This may be because the increases were too modest or because the anxiogenic drugs inhibited locomotor activity independently of DA reward systems. It is also possible, however, that these anxiogenic drugs attenuated the rewarding effects of DA neurotransmission via their stimulation of brain anxiety mechanisms. Experiments have documented electrophysiological data that delineates an anatomical basis for this type of motivational interaction. Liang, Wu, Yim, and Mogenson (1991) tested the effects of single pulse electrical stimulation of the BLA on the activity of post-synaptic rostral NAcc cells, and the modulation of this effect by iontophoretically applied DA hydrochloride into the NAcc. It was found that the BLA stimulation excited the NAcc cells (probably via NAcc GLU release), and that DA partially attenuated this excitation. Moreover, intra-NAcc microinjections of CCK completely abolished the DA-mediated attenuation of the NAcc excitation induced by the BLA stimulation. In a similar study, Yim and Mogenson (1991) found that VTA electrical stimulation attenuated BLA-stimulation-induced excitation of NAcc cells. Proglumide, a nonspecific CCK receptor antagonist, enhanced the inhibitory effects of the VTA-stimulation. As in the first experiment, the iontophoretic application of DA produced a similar effect to the VTA stimulation, and microinjected CCK8S suppressed the DA-mediated
attenuation of BLA-stimulation-induced excitation of NAcc cells. Incidentally, Hp stimulation has also been observed to excite NAcc cells, with a similar attenuation of this effect by mesolimbic DA excitation (Yang & Mogenson, 1984). Also CCK-8S microinjections into the Hp have produced decreases in lateral hypothalamic BSR rates (Heidbreder, Gewiss, De Mot, Mertens, & De Witte, 1992). CCK induces Hp pyramidal cell excitation (Dodd & Kelly, 1981), and Bradwejn and de Montigny (1984) reported that BZs antagonize the CCK-induced activation of Hp cells.

These experiments demonstrate that excitation of BLA and Hp cells can function in opposition to the post-synaptic effects of mesolimbic DA release, and under similar circumstances CCK8S can also act to attenuate the effects of DA on rostral NAcc cells. These results, therefore, lend some support to the hypothesis that anxiety can attenuate the behaviourally activating effects of stress-induced mesolimbic DA release.

It is hoped that detectable neurochemical changes in response to the i.v. CCK4 challenges proposed in this study will provide valuable insights into the neurophysiological mechanisms underlying anxiety and panic.
Materials and Methods

Subjects

The subjects utilized were 10 male adult albino rats of the Wistar strain (Charles River, Quebec, Canada), weighing 300-350 g at the time of surgery. They were individually housed within clear Plexiglas® cages lined with betachips in a temperature- and humidity-controlled environment, with ad libitum access to Purina rat chow pellets and water. The lighting was automatically maintained on a 12hr:12hr reversed schedule (0800 hrs. lights off; 2000 hrs. lights on) throughout the duration of the experiment. The animals were given at least one week to acclimatize to the housing conditions prior to surgery.

Surgery

Rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and subsequently administered atropine sulfate (0.5 mg/kg i.p.) to prevent respiratory depression during surgery. Once anesthetized, the rats were implanted with indwelling i.v. catheters exteriorised at the back between the scapulae.

The catheters were constructed of plastic-coated stainless steel guide cannulae which were protected at the base by smoothly hardened dental acrylic attached to 2x2cm Bard® Marlex® Mesh. The tubing which extended from the cannula into the vein consisted of Polyethylene (PE50) supported by silastic adhesive, followed by PE10 and flexible silastic tubing. Immediately prior to surgery, the catheters and surgical instruments were thoroughly disinfected with 2% glutaraldehyde, followed by 70 % ethanol, and finally rinsed in a distilled water and Dettol solution. All catheters were implanted into the right jugular vein with the silastic tubing extending 3.0 ± 0.2 cm towards the right atrium of the heart. Following the catheter implantation animals
were flushed with 0.1 mm of a 50 UI/ml heparin-saline solution (repeated daily to maintain patency) and immediately placed in a stereotaxic apparatus equipped with a CMA Kopf Adapter and Connecting Rod attached to a probe guide clip. The incisor bar was set at approximately -3.5 mm to produce a level skull surface and rats were then implanted unilaterally with probe guide cannulae (CMA/12) aimed directly above the NAcc according to the following coordinates relative to bregma (obtained from Paxinos & Watson, 1982): AP +1.4 mm; ML +/- 1.7 mm; DV - 6.7 mm. Following surgery animals were injected with Penlong antibiotic (1.5 ml/kg i.m.) to prevent infection. During the ensuing days, the rats were handled, cleaned, and catheters flushed daily. At least one week was allowed, and full recovery ensured, prior to testing.

Microdialysis

Microdialysis sampling was performed using CMA/12, 2.0 mm length probes constructed with a Polycarbonate-polyether copolymeric dialysis membrane with a molecular weight cut-off of 20,000 Dalton--effective to 5,000 Dalton (0.5 mm diameter) and a stainless steel shaft (0.64 mm diameter). At approximately 2000 hrs the night before testing, rats were sedated through the i.v. catheter with 2 mg of Pentothal, a fast-acting barbiturate, flushed with Heparin, and the prepared probe was then inserted into the guide. The rat was hooked up to the CMA/120 System for Freely Moving Animals (complete with a liquid swivel assembly and counter-balance arm), placed in a large clear plastic observation bowl, and given ad libitum chow pellets and water with the lights on. Overnight the probe was perfused continually with an artificial cerebrospinal fluid (aCSF) solution at 2.0 μL/min using a CMA/102 pulse-free microdialysis pump. The aCSF solution comprised of 140 mM NaCl, 3 mM KCl, 2.0 mM CaCl₂, 1 mM MgCl₂, 1.2 mM Na₂HPO₄, 0.27 mM NaH₂PO₄, and 7.2 mM glucose, all dissolved in de-oxygenated, milli-Q
filtered, distilled H₂O at a confirmed pH 7.4 ± 0.1 (Sharp et al., 1987). The aCSF was mixed fresh daily and gradually warmed to room temperature before using for perfusion through the dialysis probe.

After overnight perfusion, the flow rate was increased to 3.0 µL/min at 0800 hrs for the duration of the experiment, and the light level was reduced. For the ensuing two hours, the neurochemical environment around the probe was allowed to further equilibrate before commencing the drug testing phase of the experiment.

Throughout the study, samples were collected with a CMA/142 Microfraction Collector. Separate samples were collected every 10 minutes into plastic vials containing 6.0 µL of 0.1 N HCl to prevent oxidation, leaving a total volume of 36 µL per sample. After collection, each sample was capped, double-labeled, and placed onto dry ice. Samples were then stored in a deep freezer (-75 °C).

**Drug Administration and Behavioural Observation**

During the testing phase, animals were injected with bolus i.v. infusions (0.5 ml in under 30 sec.) of CCK4 (0, 1, 5, & 10 µg/kg) followed by 0.1 ml of the Heparin-saline solution. Injections times were adjusted to account for the delay in the flow of the perfusate from the probe membrane to the collection vial so that the beginning of the subsequent sample would coincide with the instant of the onset of the drug infusion. Animals received the CCK4 doses in a counterbalanced order and two hours separated the onset of each infusion. The average of the samples from the hour prior to each infusion served as a baseline for each respective neurochemical within the samples following the challenge.
After each CCK4 infusion, the experimenter timed and recorded the emitted behaviours of the subject. The behaviors recorded were timed according to the following mutually exclusive categories: freezing/hypervigilance, grooming, exploration, rearings (count), eating, drinking, resting, and sleeping. These observations were not tabulated by the experimenter while blind to the drug condition, and thus do not constitute a part of the present formal report. However, the results of these observations formed a basis for the behavioural experiments conducted with the same animals subsequent to the microdialysis testing.

One hour following the final CCK4 challenge, the subject was again anesthetized with Pentothal to confirm catheter patency.

**HPLC-EC Analysis**

After the completion of the microdialysis phase, samples were sent for HPLC-EC analysis to Glen Baker, Ph.D. at the University of Alberta, Neurochemical Research Unit, Department of Psychiatry and Faculty of Pharmacy and Pharmaceutical Sciences. Samples were transported overnight in an insulated Styrofoam container filled with dry ice. All samples were confirmed to arrive deep frozen the following day.

After thawing, samples were divided into two parts and analyzed separately for: 1) the monoamines and metabolites, and 2) the amino acids. For the monoamines and metabolites, 30 μL of sample (held at 4°C) was used; a volume of 25 μL was injected onto the column and moved through at 0.3 ml/min. The pre-column was a Waters Symmetry Guard and the column a Waters Symmetry C18 2.1x150 mm Sum with the column temperature set at 30°C. A Waters 460 Electrochemical detector was used with the electrical potential set at 0.85 V. Mobile phase consisted of 6.56 g NaH₂PO₄, 197.2 mg SOS, and 137.7 mg EDTA dissolved in 920 ml of
distilled H$_2$O, with 80 ml CH$_3$CN added and the pH set at 2.9 with phosphoric acid. The mobile phase was passed through a 0.25 μm filter. The mobile phase delivery and sample management was automated by the Waters Alliance 2690 XE system. The integrator and data management was performed with a digital 5100 computer using Waters Millennium software. The order of elution for the monoamines and metabolites was as follows: NA, DOPAC, 5-HIAA, DA, HVA, and 5-HT.

To the remaining sample, 6.0 μL of distilled H$_2$O was added before the sample was taken up by the Alliance sample handling system. This system requires 5.0 μL of the sample and adds 5.0 μL of O-Phthalldialdehyde (OPA) reagent to it. The OPA reacts with the primary amines on the amino acids to form highly fluorescent thioalkyl substituted isoindoles—OPA reagent (Pierce) was taken fresh daily (under nitrogen) from an intermediate stock solution to prevent oxidation. The sample was held in the loop for 90 sec to allow the above reaction to take place before being injected onto the column and was moved at a flow rate of 0.6 ml/min. The pre-column was a Waters μBondapak C18; the main column was a Waters Novapak C18 3.9x150 mm (4 μm) set at 30°C. The detector was a Shimadzu RF10A with a 12μL quartz flow cell (excitation λ=260 nm; emission λ=455 nm). During detection, the mobile phase constitutes 670 ml 0.2M NaH$_2$PO$_4$, 555 ml MeOH, 30 ml THF and is adjusted to pH 6.2 with 10N NaOH. The solution was filtered (0.2 μm) and degassed under a vacuum. The samples and data were managed by the Waters Alliance system and Waters Millennium software as with the monoamines and metabolites. The elution order for the amino acids was ASP, GLU, ASN, GLY, ALA, and GABA.
**Behavioural Testing**

**Activity**

Based on quantitative behavioural data from the microdialysis phase of the experiment, we proposed to test the effects of i.v. CCK4 challenges on exploratory/locomotor activity. At least one week following microdialysis testing, animals with patent catheters were tested in a dark room with activity cages (50 cm³) equipped with two infrared beams, raised 3 cm from the wire mesh floor. The activity cages were mounted with liquid swivels attached to counter-balanced arms. The liquid swivels were attached with a length of microbore tubing to 5.0 ml syringes filled with either 0 or 10 μg/kg CCK4 and polyethylene, spring-reinforced leads hanging from the swivel were screwed onto the subjects’ catheters. This setup allowed the animals to be injected quickly without handling. Immediately upon being set within the cages, rats were infused with the assigned dose of CCK4 to test for acute effects on novel environment exploratory activity.

Exactly 90 min later, rats were again challenged with their respective doses to test for locomotor effects in a familiar environment. The following day animals were tested with the opposite dose according to an identical protocol. Activity testing was conducted between 1500-1700 hrs.

Movement, quantified by infrared beam breaks, was recorded on 286-PC computers set up outside the testing room, which were equipped with custom-made software for monitoring total beam breaks and crossovers (consecutive breaks of front and back infrared beams). The activity data was organized into 10 min bins and calculated as percentage baseline scores (for the second CCK4 challenge) where the average crossovers for the 30 min preceding the infusion served as the baseline for the subsequent 30 min (3 x 10 min bins). The resultant scores were subjected to statistical testing.
Acoustic Startle Response

At least five days following activity testing, animals with patent i.v. catheters were tested for anxiety-related behavioural effects resulting from bolus i.v. CCK4 infusions. The capacity for CCK4 to potentiate the acoustic startle response was tested within sound attenuated MedAssociates® Startle Chambers hooked up with microbore tubing attached to a 5.0 ml syringe outside the chamber (similar to the activity testing setup) so that during the testing session rats could neither see nor hear the experimenter during injections. Rats were placed in wire rod cages that were screwed onto to an accelerometer platform. The platform was wired to a 486-66Mhz DOS/Windows3.1-based computer with Med-PC Startle Software designed to control the presentation of acoustic startle stimuli and to record the parameters of the startle response. The startle session consisted of 5 min habituation to the testing environment (with 70 dB white noise and dim red lighting) followed by 6 x 50, 5 msec white noise acoustic stimulus presentations, consisting of 100 and 120 dB intensities given in random order (with the restriction that there be 25 of each intensity within each group of 50 stimuli). Stimuli were presented according to a variable interval 30 ± 5 sec schedule.

After the initial 50 habituation stimuli, all subjects were separately administered two priming infusions followed by 50 stimuli, and then three CCK4 challenges of equal volume (one ≈0.5 ml challenge every 50 stimuli) according to the following schedule: 1) Vehicle, 2) 10 μg/kg, 3) 5 μg/kg. Testing was conducted between 1300-1900 hrs.

The 120 dB results from the startle testing procedure were calculated as percent baseline scores, where (for a given subject) the average startle amplitude for the 7, 120 dB stimuli immediately preceding a CCK4 challenge formed the baseline for the first 5 post-injection stimuli
of the same intensity. The timings for the baseline and test groups of stimuli were chosen to minimize carryover effects (≈7 min baseline), and to isolate the hypothesized anxiogenic effects of the i.v. CCK4 (based on pilot work and behavioural observation during the microdialysis testing). The resultant 5 response/baseline (%) scores for each animal, at each dose (N=90) formed the basis for the statistical testing described later in the data analysis section.

**Histology**

Following the above experiments, all rats were sacrificed and perfused with 0.9% saline and 10% Formalin. Brains were removed and stored overnight at 4°C in a 10% sucrose/formaldehyde solution. Brains were sectioned (40 μm) at -15°C and mounted onto gelatinized slides. Placements were judged according to Paxinos and Watson (1982) and injection locations within subregions of the NAcc were noted but not discriminated within the analysis.

**Drug Preparation**

For all experimentation, CCK4 (Sigma) was dissolved in de-oxygenated, milli-Q filtered, distilled H₂O also with 0.9% NaCl and 0.1% sodium metabisulphite (BDH) to prevent oxidization. The final drug solutions were stabilized at pH 7.0 ± 0.1. The sodium pentobarbitol (Somnotol, DCM) used for surgical anesthetization was obtained in solution. Pentothal was dissolved in a stock H₂O solution immediately prior to usage. Atropine sulfate was dissolved in 0.9 % saline prior to the commencement of the surgical phase of the experiment.
Data Analysis and Statistics

The results of the HPLC analysis were utilized to calculate the effects of CCK4 challenges using percent baseline (%BSL) levels of several neurochemicals as dependent variables. In all cases, the average of the detection results for the hour prior to a CCK4 challenge was used a baseline for each of the subsequent sample scores for the ensuing four 10 min bins. The resultant scores were then represented as %BSLs and statistically analyzed. Additionally, the %BSL scores for DA were represented as a ratio of the corresponding %BSL scores for DOPAC, HVA, and GLU, and all response variables involving DA were further subjected to a \( \log_{10}(Y+1) \) transformation to remedy heterogeneity of variance. Finally, separate analyses were performed to isolate averaged dose effects on the above dependent measures by using the average of the entire 40 min following a CCK4 challenge as a percentage of the preceding 60 min baseline.

Statistical analyses were performed using Statistica 1997. Additionally in certain unbalanced cases, the data was re-evaluated with PROC GLM in SAS to compare the Type I and Type III Sums of Squares as an assessment of the degree of factor confounding resulting from cell unbalancing.

Microdialysis time by dose data were analyzed using a two-way analysis of variance (ANOVA) with both Time and Dose as repeated measures factors. Averaged Dose data alone (0-40 min averages as outlined above), was additionally analyzed with a one-way repeated measures ANOVA. Appropriate pairwise comparisons of means were performed with Tukey’s Honestly Significant Difference (HSD) post hoc test, which controls for the Familywise error rate, and also with Duncan’s post hoc test, which is a more powerful but less stringent test for differences between means.
Activity testing data for behaviour following the 90 min infusions was tested with a two-factor (DOSE x TIME), fully within-subjects ANOVA design. Additionally, planned vector comparisons between drug doses at each time point were performed.

Acoustic startle data was similarly analyzed according to a two-factor (DOSE x STIMULUS#) repeated measures ANOVA design. Significant main effects or interactions were tested for post hoc mean differences with Tukey's HSD test.
Results

Microdialysis

The histological analyses revealed that 5 of the 10 rodents had probe placements directly within the NAcc. Another subject was found to have the probe placement in the very caudal tip of the NAcc, at the border between the NAcc and the ventral pallidum. The data from this 6th animal was included within the statistical analysis, and displayed dose-responsive effects similar to the definitive NAcc placements. DA sampling from one of the above animals with a NAcc probe placement yielded no detectable DA levels except briefly following the 10 μg/kg dose of CCK4. This animal was removed from the statistical analyses involving DA.

According to a two-way ANOVA, the low and high CCK4 doses (1 and 10 μg/kg) produced significant decreases in NAcc GABA levels [$F_{3,15}=3.95$, $p < 0.05$], and non-significant trends towards a dose-dependent increase in DA:DOPAC ($p = 0.1$), and DA:HVA ($p = 0.1$) ratios. However, there were no significant time effects or dose-time interactions. The separate one-way ANOVA for CCK4 dose revealed significant increases in NAcc DA:DOPAC [$F_{3,11}=4.57$, $p < 0.05$] and DA:HVA [$F_{3,11}=4.54$, $p < 0.05$] ratios, and a significant decrease in NAcc GABA levels [$F_{3,15}=4.15$, $p < 0.05$]. There was also a non-significant trend towards an increase in DA levels alone (see Figures 1-3).

Mean comparisons for the effects of the four CCK4 doses on GABA revealed significantly higher GABA levels at the vehicle dose compared with the 1 μg/kg CCK4 (Tukey’s HSD) and 10 μg/kg (Duncan’s) doses. This GABA decrease was not apparent at the intermediate CCK4 dose (5 μg/kg). Mean comparisons between CCK4 doses (one-way ANOVA) on DA:DOPAC ratios revealed an increase at the high dose compared with the low (Tukey’s HSD) and vehicle (Duncan’s) doses. Similar effects are apparent with DA:HVA ratios. No other effects were
statistically significant, although there were similar trends for CCK4-induced increases of DA (p = 0.06), and DA:GLU ratios (p = 0.08) apparent at the high dose.

Finally, following CCK4 infusions, there was an immediate but insignificant, short-lived trend towards an increase in NAcc GLU levels. This trend was only weakly detectable in two or three of the animals tested (depending on the dose).

**Behaviour**

**Activity**

CCK4 at the 10 μg/kg dose produced a significant decrease in percent baseline activity relative to vehicle when injected following 90 min of exposure to the boxes. There was a significant effect of CCK4 dose \([F_{1,4} = 8.24, p < 0.05]\). The planned vector comparisons revealed that this difference was most prominent during the first 10 min following the infusion \([F_{1,4} = 8.44, p < .05]\), and was not statistically significant during the second and third 10 min intervals (See Figure 4). Importantly, there were no visible effects of CCK4 on novel exploration. That is, only the second infusion produced effects on the number of crossovers (See Figure 5).

**Acoustic Startle Response**

CCK4 was found to significantly potentiate baseline average startle amplitudes (see Figure 6) in response to 120 dB stimuli. The effect of dose \([F_{2,10} = 5.11, p < .05]\) was due to a significant potentiation of startle at the 10 μg/kg dose compared with the vehicle infusion (p < .05). The 5 μg/kg dose was increased with marginal statistical significance (p = 0.055) over vehicle (Tukey’s HSD). There was no apparent effect of the stimulus number, nor was there significant non-additivity between the effects of dose over different stimulus presentations. The CCK4 dose effect is only apparent when the first five stimuli are used. An analysis including the first 10 stimuli did not yield a significant dose effect.
**Discussion**

In awake rodents, microdialysis sampling following i.v. infusions of CCK4 revealed significant, dose-dependent increases in the ratios of DA:DOPAC, and DA:HVA within the NAcc. A similar increase in DA levels alone was strongly indicated, although the increase was not statistically significant at the 5% chance cut-off level for Type I Errors. Although the dose by time interaction was not statistically significant, the timing (i.e., onset) of the dose-dependent DA:DOPAC increases are of key importance for the evaluation of these results (see Figure 7), and will be used for later discussion in concert with the behavioural findings. In addition to the DA-related neurochemical data, i.v. CCK4 also produced a weak decrease in NAcc GABA levels. This decrease, relative to vehicle, was only apparent at the low and high CCK4 doses. The vehicle alone appeared to produce a mild increase in GABA levels.

As a consequence of the quantitative behavioural observations completed during the microdialysis testing phase of the investigation, i.v. CCK4-induced behaviour from two distinct paradigms was evaluated. First, indications of freezing and hypervigilant behaviours prompted the study of the influence of CCK4 on horizontal activity. It was found that 10 μg/kg CCK4 attenuated activity relative to the vehicle control: a) after animals had been exposed to the activity cages for 90 min, and b) 90 min following a CCK4 infusion given immediately upon introduction to the novel testing environment. Importantly, the initial CCK4 infusion produced no apparent effects on novel exploration.

Second, anxiety-related observations of piloerection, defecation, heightened arousal, and jumpiness prompted the final experiment designed to test the fear-potentiating capacity of i.v. CCK4 by using acoustic startle measurements. It was found that during a brief 5 min period following 5 and 10 μg/kg infusions, that percent baseline average startle responses were elevated
in comparison to vehicle control infusions in the same animals. As with the activity data, this result was only apparent following an initial CCK4 infusion that failed to produce an obvious potentiation of the startle reflex. It was noted that following the CCK4 challenges, upon removal from the startle chambers, the rats appeared extremely anxious. This was indicated by observations of piloerection, excessive defecation/urination, freezing, and a markedly crouched posture. These observations were similar to the CCK4-induced behavioural responses during the microdialysis testing phase and to the CCK4-induced behaviours in monkeys (Palmour, Ervin, Bradwejn, & Howbert, 1991). The following discussion will elaborate upon the implications and complexities regarding these neurochemical and behavioural results.

**Dopamine : DOPAC Ratio**

The DA:DOPAC ratios are of particular interest since this measure in rodents can be interpreted to indicate the availability of DA in NAcc tissue. If DA increases are observed in the absence of significant increases in its metabolites, this implies that the DA is being released at a greater rate than it is being enzymatically degraded. In these data, there is no substantial evidence to suggest that DOPAC or HVA levels are increased. It is not until a later point in time that DOPAC and HVA levels show a trend towards an increase. As an aside, the concentration of recovered DA at baseline was of the order of 0.5 pg/μL, in contrast to 50 pg/μL concentrations of DOPAC and HVA. The recovered concentrations of DA and the metabolites obtained in the present study are comparable with that of other published microdialysis data (Robinson & Camp, 1991).
The animals used for behavioural testing were in most cases used for microdialysis testing in response to i.v. CCK4 challenges within the BLA and the PFC. Only one animal from the NAcc neurochemical data was also used for behavioural testing. Moreover, it is important to emphasize that the behavioural experiments were not performed simultaneously with the *in vivo*, awake microdialysis testing. In spite of the lack of correspondence between the subjects used for the NAcc microdialysis and behavioural experiments, the present discussion will briefly compare the time courses of the intra-NAcc neurochemical changes with the behavioural effects induced by i.v. CCK4. It is apparent from comparisons between the time courses for the CCK4-induced behaviours (0-10 min post-infusion) and the neurochemical effects (10-30 min post-infusion), that the percent baseline increases in DA:DOPAC do not temporally correspond with the behavioural effects of the i.v. CCK4. In fact, the DA increases appear to follow both the activity attenuation and the startle potentiation—both of which are no longer apparent more than 10 min following the challenge.

In view of the temporal differences between the behavioural and neurochemical effects, it is important to emphasize that these neurochemical measurements are correlational in nature, and thus can not be clearly interpreted as direct consequences of the manipulation. These changes could represent a delayed compensatory neurophysiological response to other more immediate events following the CCK4 infusion. In general, changes in local neurochemistry cannot be interpreted as causal determinants of observed behavioural changes. These data also present a further paradox: Following the short decrease in activity, the delayed DA:DOPAC increase is not associated with a simultaneous increase in horizontal activity—a behavioural effect that typically accompanies elevated NAcc DA. Similar neurochemical and behavioural effects from beta-
carboline anxiogenic challenges have also been reported (McCullough & Salamone, 1992). Therefore, these DA-related increases are interpreted to reflect a secondary neurophysiological response to the primary effects of CCK4, and these primary anxiogenic-like effects of CCK4 are likely associated with neurophysiological responses outside of the NAcc synaptic region. Moreover, given the low recovery of DA, GLU, and 5-HT (not detectable) from the microdialysis samples, the present experiment would not likely have been able to detect decreases in these neurochemicals. This has important implications for the interpretation of the acute i.v. CCK4 effects.

**Acute I.V. CCK4 Effects**

It is clear that the local effects of CCK_B receptor stimulation within the NAcc are associated with a decrease in DA neurotransmission (Ladurelle et al., 1993; Marshall et al., 1991; Studler et al., 1986; Voight et al., 1985; 1986). Therefore, the neurochemistry of local NAcc CCK-DA interactions would lead to the hypothesis that the acute effects of i.v. CCK4 would lead to a decrease in NAcc DA. Since the behavioural effects of NAcc CCK_B receptor stimulation are consistent with a decrease in reward-related behaviour (Bush et al., 1996; Higgins et al., 1994; Josselyn & Vaccarino, 1995; Vaccarino & Rankin, 1989; Vaccarino & Vaccarino, 1989; for review Vaccarino, 1994), it is critical to point out that the immediate behavioural effects of i.v. CCK4 are completely consistent with this literature of behavioural findings. In fact, the lack of immediate observable DA increases during the time point at which anxiety (startle data) and hypolocomotion were apparent supports an important possibility. Specifically, the effects of i.v. CCK may give rise to reward-related behavioural results that are consistent with anxiety. Thus the anxiogenic effects of i.v. CCK4 are behaviourally consistent with the reward-attenuating
effects of CCK4 in the NAcc. This highlights the hypothesis that the distinct psychological effects of CCKB receptor activation within different brain regions cooperate to motivate an organism in a common final behavioural direction. This theoretical possibility is introduced from the point of view of addiction in a recent review by Vaccarino (1995). Here, the indicated decrease in mesolimbic DA-mediated behaviour may have been associated with an undetectable rapid decrease in acute NAcc DA levels. The present methodology, which is limited by threshold detection levels and potentially insufficient temporal resolution, may have failed to detect rapid i.v. CCK4 effects.

NAcc GABA Effects

Although the decreases in GABA at the low and high CCK4 doses were statistically significant, and are consistent with the literature on the general role of GABA in anxiety (although the connection between NAcc GABA and anxiety is less certain), several aspects of this data suggest that this may not be a CCK4-induced effect. Unlike the DA-related data, the GABA shifts do not appear to be temporally associated with the CCK4 challenges. Moreover, these data are not systematically dose-related since the intermediate CCK4 dose is not significantly different from the vehicle condition. Specifically, the vehicle control injection is associated with an elevated percent baseline GABA level. At the low and high doses the magnitude of the percent baseline decreases are not large. Thus, given the interpretive complications with this data, it is suggested that the GABA changes perhaps are not reflective of the acute effects of CCK4 and may in fact be due to complications with respect to the experimental design that will be subsequently be elaborated upon.
Complexities of Interpretation

Order Effects.

There are several potential complexities regarding the theoretical significance of these results. For the microdialysis testing, CCK4 dose was a repeated measures factor, where the injections were counterbalanced with respect to the order of administration. However, since the final number of subjects was not a multiple of the number of doses, the ordinate position of each dose administration could not be completely balanced. Consequently, certain confounds including circadian rhythms, priming, and even subtle carryover effects could have affected the results. Circadian patterns in particular could have produced the type of results obtained for GABA, where cyclical baseline shifts could produce changes that would be apparent across a dose level. This is particularly likely since, as noted above, the GABA changes do not correspond with the onset of the injections.

Detection Levels.

The microdialysis data was also affected by low detection levels for the monoamines. This produced less than ideal baseline stability from one sample to the next for DA. These problems could be influenced by the slightly high flow rate necessary here, which would have the effect of decreasing relative recovery levels. The need to divide the samples to test for the monoamines and the amino acids imposed this difficulty. The variable baseline could also be potentially remedied by adding a monoamine oxidase inhibitor to the aCSF to reduce enzymatic degradation and oxidation during the time it takes for the perfusate to travel from the probe to the sample vial.

Priming or Conditioning Effects.

Another more general problem is highlighted in the behavioural data. It was found that CCK4 produced very short-lived behavioural effects, and these were observed only in a
habituated environment after an earlier CCK4 priming infusion was given. This was apparent with both the activity and the startle testing. In fact, behavioural observations during the microdialysis testing also supported the notion of a priming or conditioning effect. Thus conditioning, habituation, and/or priming effects may be critical for certain CCK4-induced changes. Given the sample size, there are insufficient degrees of freedom to analyze the order-related effects from these experiments; moreover, the CCK4-related effects on acoustic startle amplitudes are inextricably confounded with order and time-dependent factors. There are several possible interactions between these factors and the effects of CCK4 that need to be thoroughly examined. The ensuing discussion will speculate about possible theoretical implications introduced by these results.

Possible Implications for the Order-Related Effects of CCK

As noted, the effects of CCK4 on activity and acoustic startle were only apparent after a previous administration of CCK4. Although the nature of this finding is unclear, and the design of the present study does not sufficiently distinguish between priming, conditioning, carryover, habituation, and order effects, the fact that this phenomenon was found in both behavioural paradigms begs further inquiry. First of all, the duration of the CCK-induced behavioural changes appears to be very short. In the case of the startle paradigm, beyond the first five 120 dB stimuli (about 5 min), the potentiation of startle is no longer apparent. Secondly, the magnitude of these effects is not large, and was only revealed after a careful analysis of each individual's behaviour. Therefore, it is possible that these behavioural (and neurochemical) effects are simply difficult to detect. It is alternatively possible that the second infusions were identifiably efficacious because there were subtle carryover effects from the previous CCK4 infusion; however, given the short
duration of any observable behavioural effects, this explanation is probably insufficient.

The hypothesis to be considered here is that acute pre-exposure to a CCK4 infusion somehow sensitizes the animal to a subsequent CCK challenge. Either conditioning or physiological priming could subserve this effect. Recent data suggests that CCK4 microinjections into the BLA potentiate acoustic startle (Vaccarino, Arifuzzaman et al., 1997). Pentagastrin microinjections have also been reported to reliably potentiate startle through activation of this region (Frankland et al., 1996; 1997). Interestingly, recent work from Davis and colleagues dissociates the role of the amygdala from that of the bed nucleus of the stria terminalis (BNST; Lee & Davis, 1996; McNish, Gewirtz, & Davis, 1997; Walker & Davis, 1996; 1997), where the amygdala is implicated in the control of cue- or context-dependent anxiogenesis (conditioned fear), and the BNST is implicated in the control of state-dependent anxiogenesis (general anxiety).

There is important evidence that highlights the importance of CCK in the mediation of conditioned fear. In this experiment, fear-potentiated startle (enhanced startle in response to the presentation of a shock-paired conditioned stimulus) was selectively attenuated by the CCK$_B$ antagonist, L-365,260 (Josselyn et al., 1995). Since CCK$_B$ receptor stimulation potentiates startle in the BLA, the region implicated in conditioned fear (Davis, 1992), it is possible that CCK endogenously mediates or modulates the neurophysiological mechanisms that subserve cue-specific fear and fear-learning. This speculation gives introduces the hypothesis that systemic CCK4 administration not only induces certain acute fear symptoms, but also directly subserves certain physiological mechanisms involved in fear conditioning. Thus CCK4 injections that are given in the same environmental conditions in which CCK4 has been previously administered, could be expected to produce enhanced (and more general) fear-related behaviours than a single acute CCK4 challenge. Essentially, both the physiological symptoms and the environmental
circumstances associated with the administration of CCK would become conditioned cues for fear.

There are potential implications that could arise from this hypothesis. Given the colocalization of CCK with DA in mesolimbic/mesocortical VTA cells, combined with the role of DA in reinforcement, it is possible that CCK and DA interact to mediate motivational learning and motivational behaviour within both the BLA and the NAcc. Based on anatomical evidence, CCK is very likely to be co-released with DA from VTA cells projecting both to the BLA and the NAcc (Hökfelt, Rehfeld et al., 1980). The function of CCK and DA in motivational learning with both positive and negative valence is of particular interest, since these interactions could be intricately involved with drug abuse phenomena (Bush, DeSousa, & Vaccarino, 1997; Vaccarino, 1995), as well as panic disorder-induced phobias. It is recommended that further studies be designed to test behavioural responses to CCK4 challenges following priming CCK4 infusions given either in the test environment or in a separate, distinct environment. Experiments of this nature could determine the cue-dependency of certain observable anxiogenic effects of CCK. Similarly, it may be possible to isolate the conditioned and unconditioned behavioural effects of CCK. At the very least the data presently obtained provides evidence for an acute, short-lived anxiogenic-like effect resulting from i.v. CCK4 under certain conditions.
Figure Captions

Figure 1: Effect of i.v. CCK4 on DA levels in the NAcc.

Figure 2: Effect of i.v. CCK4 on DA:DOPAC ratio in the NAcc. (* p < 0.05 with Tukey's HSD relative to vehicle).

Figure 3: Effect of i.v. CCK4 on DA:HVA ratio in the NAcc. (* p < 0.05 with Tukey's HSD relative to vehicle).

Figure 4: Effect of i.v. CCK4 on activity. (Main effect of dose, p < 0.05; * p < 0.05: a priori comparison).

Figure 5: Time course for the effects of i.v. CCK4 on activity (* p < 0.05 with Tukey's HSD). Arrows indicate time point of injection.

Figure 6: Effect of i.v. CCK4 on the acoustic startle response (* p < 0.05 with Tukey's HSD relative to vehicle).

Figure 7: Time course for the effects of i.v. CCK4 on NAcc DA:DOPAC ratios. Dose labels indicate first 10 min bin following infusion. Arrows indicate time point of injection.
References


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Figure 1: Effect of IV CCK4 on NAcc Dopamine
Figure 2: Effect of IV CCK4 on NAcc DA:DOPAC Ratio

LOG (DA:DOPAC) % Baselines

CCK4 Dose (µg/kg)

0
1
5
10

0 0.2 0.4 0.6 0.8 1 1.2
Figure 3: Effect of IV CCK4 on NACeDA: HVA Ratio

LOG(DA:HVA)

% Baselines

0.2
0.4
0.6
0.8
1
1.2

CCK4 Dose (11/6/kg)
Figure 4: Effect of IV CCK4 on Activity
Figure 5: Effects of IV CCK4 (10 μg/kg) on Activity
Figure 6: Effect of IV CCK4 on the Acoustic Startle Response

Average Startle Amplitude (% Baseline)

CCK4 Dose (μg/kg)

10  5  0
Figure 7: Time Course for the Effects of IV CCK4 on NAcc DA:DOPAC Ratio