Cocaine effects on striatal dynorphin and CART neuropeptides: association to mood disorder

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Stockholm 2003
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Published and printed by Karolinska University Press
Box 200, SE-171 77 Stockholm, Sweden
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ISBN 91-7349-515-8
To be continued
ABSTRACT

People take cocaine to elevate mood, but with repeated use and subsequent development of dependence, in paradox, a negative mood state is induced. This may be one reason for the strong comorbidity between cocaine dependence and mood disorders. A common substrate implicated in both disorders is the neurotransmitter dopamine. Alterations of the dopamine system lead to neuroadaptations, such as modulations of gene transcription in postsynaptic neurons. This thesis work involved examination of mRNA expression of neuropeptides in dopamine-related systems and their response to cocaine administration. Specifically, the opioid neuropeptide dynorphin involved in the regulation of emotion and motor function and the novel neuropeptide cocaine and amphetamine regulated transcript, CART were studied. The focus was on the striatum, a brain region critical for limbic and motor functions, which is affected by cocaine. In addition, behavioral disturbances in relation to the comorbidity between cocaine and mood disorders were investigated.

We found CART mRNA expression in the human brain to be highly expressed in brain regions implicated in cocaine abuse, including most target regions of the mesocorticolimbic dopamine pathway and regions in the striatopallidal circuitry. These findings support a putative role of this neuropeptide in the effects of cocaine. In agreement, we found CART mRNA expression to be regulated by acute cocaine administration in the rat.

The well documented up-regulation of prodynorphin mRNA following cocaine exposure was confirmed and expanded in this thesis. We demonstrated dose-dependent and temporal elevation of the prodynorphin mRNA in the dorsal striatum of monkeys that had self-administered cocaine. The limbic-related patches/striosomes were initially more sensitive to the induction of the prodynorphin gene transcription with a progression to the sensorimotor-related matrix after long-term, high-dose, cocaine self-administration. In contrast, we found reductions of the striatal prodynorphin and dopamine D1 receptor mRNAs following 10 days abstinence from repeated cocaine injections in the rat, suggesting a long-lasting alteration in the striatonigral pathway following cocaine exposure. The observed suppressed striatal prodynorphin mRNA levels following cocaine abstinence was matched in a genetic animal model of depression, the Flinders Sensitive rat line (FSL). The FSL rats exhibited reduced prodynorphin mRNA levels in the caudal striatum during basal conditions. These results imply a low striatal dynorphin tone during a negative mood state, which may be related to psychomotor retardation.

The effects of cocaine on a depression genotype were investigated in the FSL rats. These animals acquired cocaine self-administration behavior at a similar rate as their controls, but we found a subtle reduction in cocaine reinforcement; cocaine intake was reduced at one dose in a dose-response curve. In addition, the FSL rats were low responders to novelty, which is associated with decreased vulnerability to addictive drugs. In contrast, the response to repeated cocaine administration indicated greater sensitivity to behavioral sensitization, as demonstrated by enhanced stereotyped behavior. Despite apparent motor differences the FSL rats showed a similar dopaminergic response in the nucleus accumbens shell to repeated cocaine administration, as measured by in vivo microdialysis. Taken together, the depression genotype was associated with behavioral differences in the response to cocaine. However, definite conclusions on the reinforcing efficacy of cocaine in the FSL rat will require further studies.
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<tr>
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<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CREB</td>
<td>cAMP response element binding protein</td>
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<tr>
<td>CRF</td>
<td>Corticotropin releasing factor</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
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<tr>
<td>DPM</td>
<td>Disintegration per minute</td>
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<tr>
<td>DSM IV</td>
<td>Diagnostic and Statistical Manual of Mental Disorders IV</td>
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<tr>
<td>DYN</td>
<td>Dynorphin</td>
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<td>FR</td>
<td>Fixed ratio of reinforcement</td>
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<td>GABA</td>
<td>Gamma-aminobutyric acid</td>
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<tr>
<td>G-protein</td>
<td>Guanine nucleotide binding-protein</td>
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<td>HPLC</td>
<td>High performance liquid chromotography</td>
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<td>i.p.</td>
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<td>Intracranial self-stimulation</td>
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<td>In situ hybridization histochemistry</td>
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<td>L-DOPA</td>
<td>Dihydroxyphenylalanin</td>
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<td>mRNA</td>
<td>Messenger ribonucleic acid</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>PDYN</td>
<td>Prodynorphin</td>
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<tr>
<td>RPA</td>
<td>Ribonuclease protection assay</td>
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<tr>
<td>SEM</td>
<td>Standard error of the means</td>
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<tr>
<td>SNC</td>
<td>Substantia nigra compacta</td>
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<tr>
<td>SNr</td>
<td>Substantia nigra reticulata</td>
</tr>
<tr>
<td>VTA</td>
<td>Ventral tegmental area</td>
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1 INTRODUCTION

Cocaine is derived from the coca-plant (Erythroxylum Coca) and is one of the most potent addictive drugs used by man. The psychoactive effects of the drug were first experienced by South American Indians who already 3000 BC were chewing coca leaves. E. Coca was brought to Europe in the 16th century, and the active substance cocaine was extracted from its leaves in 1861. In 1870, Vin Mariani (Coca wine) was produced and gained wide popular acceptance in the western countries. The medical world recognized the effects of cocaine in the 1880s (e.g., see Fig. 1). Furthermore, the psychologist Sigmund Freud declared cocaine as a safe and useful medicine that could cure depression and sexual impotence as well as morphine addiction (Freud 1884). Cocaine gained further popularity in 1886 when John Pemberton included it as the main ingredient in his new soft drink, Coca Cola. The abuse potential and addictive property of the drug was recognized in the late 19th century and public pressure forced the removal of cocaine from Coca Cola in 1903. As a medical drug, cocaine is still used as an anesthetic and vasoconstrucive agent, especially suitable for nose, throat and eye surgeries (for history see Karch 2002).

![Figure 1. Toothache drops advertisement from 1885](image)

After extraction from the coca leaves cocaine is most often precipitated as cocaine hydrochloride. This powder is usually "snorted" (nasal inhalation) or, in heavy users, dissolved in water and injected intravenously. Snorting cocaine has its limitations due to vasoconstriction of the nasal mucosa and slow onset of action (minutes). In contrast, smoked cocaine has an extremely rapid onset (10-15 s) comparable to, and even faster than, intravenous use. In order to smoke cocaine, acidic cocaine hydrochloride must be converted to a chemical base. The product, freebase cocaine or "crack", looks like small lumps with the texture of porcelain. When heated, this form of cocaine makes a crackling sound (hence the name), and it is easily vaporised for inhalation (see Chychula & Okore 1990).

In the USA cocaine is one of the most commonly abused heavy drug but in Sweden, cocaine use has been limited due to its poor availability and subsequently high price. Instead amphetamine, a synthetic drug that belongs to the same class of drugs, the
psychostimulants, is the most commonly used drug after marijuana (i.e., hashish). However, over the past few years the availability of cocaine has dramatically increased in Sweden, as evident from the number of cocaine-related deaths and the police and customs drug seizures.

1.1 COCAINE DEPENDENCE

Although drugs that are abused are highly addictive, not all individuals become dependent after using these substances. Genetic, social, and environmental factors all influence the propensity to develop substance dependence. It is therefore important to distinguish between substance use, abuse and dependence. Substance use refers to a controlled drug intake for non-medical purposes (e.g., social drinking). According to the American classification system for psychiatry disorders, Diagnostic and Statistical Manual of Mental Disorders (DSM IV; Association 1994), substance abuse is defined as controlled harmful drug intake that is continued despite negative effects (e.g., physical hazards or failure to fulfill obligations at work, school or home). Substance dependence is defined as uncontrolled drug intake in which the individual needs the drug in order to function. The diagnostic criteria for substance dependence are presented in Table 1. The development of substance dependence specifically involves tolerance, withdrawal reactions, chronic relapses, and compulsive drug intake. Tolerance refers to the need for increased amount of the drug to achieve the desired effect. Withdrawal can be manifested as physical abstinence symptoms that are characteristic for the drug or as the use of the drug to avoid these symptoms. Cocaine dependence is not associated with specific physical withdrawal reactions, such as e.g., pain and nausea, that occurs during opiate abstinence. Instead psychological withdrawal reactions that are common for most substances, such as irritability, depression, and anxiety are observed during early abstinence from cocaine.

The transition from drug use to dependence is suggested to involve neuroadaptations that are subsequently manifested as tolerance, withdrawal reactions, and compulsive drug-seeking behavior. Initial drug use mainly involves positive reinforcement, in which the drug effects are pleasurable. At later phases the drug use may be continued in order to alleviate the withdrawal effects it produces, referred to as negative reinforcement. The positive reinforcing effects of cocaine are expressed as initial “rush”, euphoria, gregariousness, alertness, and vigor. However, at a high dose, or following long-term use, the initial euphoria is mixed with anxiety and craving for the drug (Spotts & Shontz 1984) which often leads to compulsive drug intake in the form of “binging” (repeated high dose drug intake over a short time span). Withdrawal from long-term cocaine abuse is associated with negative mood states such as anxiety and depression (Gawin & Kleber 1989). The negative emotional state during abstinence may be the driving force for the continuation of drug use (negative reinforcement), i.e., induction of craving and subsequent relapse. These effects may be mediated through counteradaptive processes, which are neuronal regulatory attempts to normalize the alterations related to the reward stimulation. Such processes change the reward related systems from their normal operating level to a pathological state, a phenomena termed...
TABLE 1  
Diagnostic criteria for substance dependence (DSM-IV).

A maladaptive pattern of substance use, leading to clinical impairment or distress, as manifested by three (or more) of the following, occurring at any time in the same 12-month period:

1. Tolerance  
2. Withdrawal  
3. The substance is often taken in larger amounts or over a longer period than was intended.  
4. There is a persistent desire or unsuccessful efforts to cut down or control substance use.  
5. A great deal of time is spent in activities necessary to obtain the substance, use the substance or recover from its effects.  
6. Important social, occupational, or recreational activities are given up or reduced because of substance use  
7. The substance use is continued despite knowledge of having a persistent or recurrent physical or psychological problem that is likely to have been caused or exacerbated by the substance.

“allostatic” load (Koob & Le Moal 2001). In addition to counteradapative processes, sensitization processes are involved in the mechanisms underlying drug dependence (see Robinson & Berridge 2001). Sensitization is the enhanced behavioral or neurochemical response to a drug following its repeated exposure. In this mechanism, the brain circuits involved in motivation and reward becomes hypersensitized (sensitization) to drugs and drug-associated stimuli. The sensitized brain systems are suggested to mediate the reward aspect of “wanting” the drug (term incentive salience) rather than mediation of pleasurable effects, “liking” the drug. These sensitization and counteradapative mechanisms together leads to the compulsive pattern of drug-seeking behavior.

1.2 COCAINE DEPENDENCE AND MOOD DISORDER COMORBIDITY

Cocaine dependence is characterized by changes in mood. According to Kaplan and Sadock (1998), mood is defined as “a pervasive and sustained emotion that colors the person’s perception of the world”, and “common adjectives used to describe mood include depressed, despairing, irritable, anxious, angry, expansive, euphoric, empty, guilty, awed, self-contemptuous, frightened, and perplexed.” In DSM IV, the term “mood disorder” is interchangeable with “affective disorder,” which only includes depressive disorders, i.e. major depression, bipolar disorder, dysthymic disorder, substance-induced mood disorder. In this thesis, mood disorder refers to both depressive disorders and anxiety, based on the prevalence of both in cocaine dependent subjects. There is also a high comorbidity between mood and anxiety disorders (Merikangas et al. 1996; de Graaf et al. 2003).

Epidemiological reports support a strong comorbidity between substance dependence and mood disorders (Kessler et al. 1996; Goodwin et al. 2002). For example, in a US community sample, it was found that 32% and 24% of people with any affective or anxiety disorder, respectively, will have a substance abuse disorder at some time in their lives. Similarly there was a 34% lifetime prevalence of affective or anxiety disorders in cocaine dependent individuals (Regier et al. 1990). There are several possible explanations for this comorbidity. In addition to the cocaine-induced depression following long-term cocaine abuse, cocaine dependence may develop
secondary to a mood disorder in attempt to self-medicate (Khantzian 1985). Furthermore, there could be common genetic factors with or without environmental influences that make the individual more or less vulnerable to developing the disorders.

There is also evidence that the reinforcing properties of cocaine are altered in the presence of depression. In support of the self-medication theory, it has been reported that the subjective effects of cocaine are enhanced in cocaine abusers with depressive symptoms (Sofuoglu et al. 2001). Uslaner et al (1999) also reported a positive correlation between self-reported depressive symptoms and cocaine induced feelings of “high” in cocaine-dependent men. In agreement, depressed cocaine abusers report higher craving for cocaine and have greater perceived benefits from the drug use as compared to non-depressed cocaine abusers (Schmitz et al. 2000). Furthermore, individuals with severe major depression experienced a single exposure to the psychostimulant d-amphetamine as more rewarding as compared to controls (Tremblay et al. 2002). However, reduced “liking” of d-amphetamine has also been reported in subjects with depression symptoms (de Wit et al. 1987). Similarly, a decrease in methylphenidate-induced euphoria is evident in Parkinson patients (Cantello et al. 1989; Persico et al. 1998), suggesting that a negative mood state or reduced dopamine levels (which is observed during depression, see section 1.4.3.2) decreases the ability to experience pleasure (anhedonia).

1.3 ANIMAL MODELS

Animal models allow a possibility to experimentally study neurochemical processes in the living brain. The rodent brain anatomy has several significant characteristics equivalent to the human brain, but the non-human primate shows greater homology and is consequently a more optimal model. In this thesis, both rat and monkeys were studied. In the field of substance dependence, the uses of animal models are strongly validated since animas have been shown to self-administer most addictive drugs abused by humans. Experimental animal models of mood disorders have more limitations because affective states cannot be easily determined in animals. Nevertheless there are some useful models that have been validated by the reversal of symptoms by clinically effective antidepressants or anxiolytics.

1.3.1 Substance dependence

Experimenter administered drug injections, e.g., intraperitoneal (i.p.), intravenous (i.v.) or intracranial, is most frequently used to study the acute or chronic pharmacological effects of drugs regardless of the motivational state.

A more optimal model to study dependence is the drug self-administration paradigm in which the animal itself can control their drug intake. In the conditioned operant drug self-administration paradigm the animal is trained to press a lever to obtain a drug delivery (systemic or intracranial). The lever pressing and presentation of a “cue” light during drug delivery is paired (conditioned) to the drug delivery. If the drug is rewarding it acts as a positive reinforcer and lever pressing will be continued. In studies where rats have 24h unlimited access to cocaine, self-administration will be continued
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until starvation and death (Bozarth & Wise 1985). Different paradigms can be studied when using the self-administration paradigm. In the fixed ratio (FR) schedule of reinforcement a fixed number of lever-presses will result in the delivery of a drug infusion. When responding is stable, the animal maintains its drug plasma concentration at the same level for the entire session. Consequently, if the dose is lowered the number of lever-presses will increase, and subsequent dose response functions can be established. Distinct phases of substance dependence can be identified using the FR schedule, the initiation (acquisition) and the maintenance phase. During the acquisition phase of drug self-administration, behavior is mainly determined by the positive reinforcing effects of the drug (feelings of pleasure or euphoria), while during the maintenance phase, responding is additionally determined by negative reinforcing effects (e.g., alleviation of withdrawal symptoms).

Other models to evaluate drug reinforcement include conditioned place preference and drug discrimination (see Altman et al. 1996).

1.3.2 Mood disorder

Experimental animal studies on mood disorders are assessed in two ways. First, the expression of mood can be evaluated in specific behavioral tests. Second, there are animal models of depression in which the animals exhibit behavioral traits that resemble human depression.

1.3.2.1 Behavioral methods

The elevated plus-maze is one of the most validated and extensively used anxiety model. In this model rats are allowed to explore a novel plus-shaped maze. The maze consists of two open and two closed arms that are elevated above the ground. The model is based on a conflict between the exploratory drive and fear of elevated open areas. Normally rats prefer the closed arms of the maze; consequently, the time spent on the open arms is considered to reflect the level of anxiety in the rat. Clinically effective anxiolytics will enhance the time spent on open arms, whereas anxiogenic treatments will result in reduced exploration on the open arms (Pellow et al. 1985).

Other anxiety models are the conflict paradigm, light-dark test box, open field test, and defensive burying paradigm (see Belzung & Griebel 2001). Examples of behavioral depression models include the forced swim test, learned helplessness, behavioral despair and the anhedonia models saccharine preference and brain reward stimulation (see Porsolt 1979; McKinney 1984; Willner 1984).

1.3.2.2 Animal models of depression: Flinder Sensitive Line rats

Mood disorders are caused by neurochemical alterations in response to external or internal (e.g., hormonal) events and it is widely assumed that there is an individual vulnerability to the response of such events. Genetic factors are believed to contribute to the individual vulnerability. The Flinders Sensitive Line (FSL) rats have been proposed to be a genetic animal model of depression. Validating criteria for an animal
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model of depression are construct, face, and predictive validity. Construct validity refers to the theoretical rationale for the model, face validity refers to how well the model resembles the symptoms of depression, and predictive validity refers to how well the model responds to clinically effective antidepressants. The Flinders animal model of depression was developed based on the cholinergic hypothesis of depression. The cholinergic hypothesis was suggested after the discovery that active cholinesterase inhibitors cause depressed mood, anergia, and psychomotor retardation (Janowsky et al. 1974). It was further demonstrated that depressed humans are supersensitive to cholinergic stimulation (Risch et al. 1983). The FSL line and its control FRL (Flinders Resistant Line) were established by selective breeding of rats that were hyper- and hypo-responsive, respectively, to the anticholinesterase agent diisopropyl fluorophosphate (Overstreet et al. 1979). However, these rats not only exhibit cholinergic disturbances, but also monoaminergic abnormalities (see Yadid et al. 2000), which is in agreement with the monoaminergic theories of depression. Taken together the neurochemical disturbances observed in the FSL rats gives construct validity to the model. Regarding face validity, the FSL rats show similarities with depressed individuals in several aspects more than hypercholinergia. Human depressive signs such as disturbances in weight, sleep, psychomotor activity and stress response are mirrored in the FSL rats by reduced body weight, increase in REM sleep and reduction in time to REM sleep onset, reduced locomotor activity and increased stress reactivity (see Overstreet 1993). There is also an indication that the FSL rats exhibit signs of anhedonia (reduced ability to experience pleasure). Pucilowski et al (1993) found the FSL rats to be more sensitive to the stress-induced reduction in saccharine intake as compared to control, but no difference has also been reported (Ayensu et al. 1995). The Flinders rats fulfill the third validation criterion, predictive validity, because the enhanced immobility in the forced swim test can be normalized by antidepressants. Chronic, but not acute, treatment with several clinically potent antidepressants such as tricyclics and serotonin reuptake inhibitors, has been found to be effective, but lithium and bright light therapy were ineffective (Overstreet 1993; Overstreet et al. 1995; Zangen et al. 1997; Caberlotto et al. 1999).

Other genetic animal models of depression are the Congenital learned helplessness, Roman low avoidance, and Fawn Hooded rat strains (see Willner & Mitchell 2002), and recently the Wistar-Kyoto rat (Pare & Redei 1993). Validated nongenetic models include maternal deprivation, chronic mild stress, and olfactory bulbectomy (see Cairncross et al. 1979; Redei et al. 2001).

1.4 NEUROBIOLOGY UNDERLYING COCAINE DEPENDENCE AND MOOD DISORDERS

1.4.1 Anatomical substrates

1.4.1.1 Mesocorticolimbic dopamine system

The neuroanatomical substrate that has been most implicated in the pathophysiology of cocaine dependence is the mesocorticolimbic dopamine system and its target regions. The mesocorticolimbic dopamine pathway originates in the ventral tegmental area
(VTA) and projects to cortical and subcortical forebrain areas (Dahlström & Fuxe 1964; Ungerstedt 1971). The subcortical limbic areas include the ventral striatum, amygdaloid complex, bed nucleus of stria terminalis, hippocampus, septum, and olfactory tubercle and the cortical areas include the prefrontal, cingulate, piriform and entorhinal corticies. In monkeys and humans, the cortical dopamine projections are more widespread as compared to the rat, but the frontal corticies are most densely innervated in all species. Long-term alterations in the amygdaloid complex, cingulate cortex, and prefrontal cortex have been documented in human cocaine dependent subjects (Grant et al. 1996; Childress et al. 1999; Volkow & Fowler 2000; Franklin et al. 2002).

1.4.1.2 Striatum

Dopaminergic innervation

Another anatomical site that is partly related to the mesocorticolimbic dopamine system is the striatum. This region shows a strong interaction between the dopamine and neuropeptide systems that are highly implicated in both cocaine dependence and mood disorders. The striatum is one of the most densely dopamine- innervated area in the brain. In the rodent, the dorsal striatum receives dopaminergic innervation from the nigrostriatal pathway that originates in the substantia nigra compacta, whereas the ventral striatum is innervated by the VTA (Dahlström & Fuxe 1964; Ungerstedt 1971). In addition, the dopaminergic projections from the retrorubral area innervate both ventral and dorsal striatum. The primate has the classical nigrostriatal and mesocorticolimbic pathways, but the topographic organization of the dopaminergic neuronal populations has noted differences to the organization found in the rat. Dopamine neurons are organized into “ventral” and “dorsal tier” cell groups, in which the ventral tier projects to the dorsal striatum, and the dorsal tier includes VTA neurons and constitutes the mesocorticolimbic pathway with projections to the ventral striatum (see Lynd-Balta & Haber 1994).

Anatomy

The majority of the striatal neurons are GABAergic medium spiny neurons (95 % in the rat, 70-80% in the primate) but differ in their neuropeptide content. Most spiny neurons are projection cells that coexpress the neuropeptides dynorphin, substance P, enkephalin and/or neurotensin (Palkovitz et al. 1984; Beckstead & Kersey 1985). Dynorphin and substance P are generally found in the same cell populations. In addition to projection neurons there are GABAergic interneurons that use neuropeptide Y and somatostatin as modulatory cotransmitters. There are also large aspiny cholinergic interneurons that modulate the dopaminergic activity in the striatum (see Heimer et al. 1985).

In the rat, the dorsal striatum is referred to as the caudate-putamen and the ventral striatum as the nucleus accumbens. In the primate, the dorsal striatum is divided into the caudate nucleus and putamen, and these structures converges in the ventral striatum, nucleus accumbens. The nucleus accumbens is further divided into a core and shell
region in rodents (Heimer et al. 1990), monkeys (Ikemoto et al. 1995) and humans (Voorn et al. 1994). In the rat, the most rostral tip of the nucleus accumbens is referred to as the rostral pole that has a combination of core and shell organization (Zahm & Brog 1992a). Thus far, there is no identified “rostral pole” correlate in the primate (Meredith et al. 1996).

Functional anatomy

Functionally, the striatum is, based on its differentiated cortical glutamatergic innervation, organized in three subregions: the motor, associative and limbic striatum (see Fig. 2). In the rat, the motor striatum comprises the lateral caudate-putamen and is innervated by lateral and medial agranular corticies (McGeorge & Faull 1989). The associative striatum comprises the medial caudate-putamen and is innervated by the anterior cingulate (Groenewegen et al. 1990). The limbic striatum comprises the ventral striatum, which receives input from hippocampus, amygdala, and prefrontal areas such as orbital, infralimbic, prelimbic, and agranular insular corticies (Heimer et al. 1991).

A similar functional organization is defined in the primate striatum (Fig. 2; see Parent & Hazrati 1995). The motor striatum consists of mainly the postcommissural dorsolateral putamen and dorsolateral caudate nucleus; it is innervated by primary motor cortex, premotor cortex, supplementary motor area and postarcuate premotor area. The associative striatum consists of precommissural dorsal putamen and most of caudate nucleus; it receives input from associative cortical areas such as medial and dorsolateral prefrontal corticies. The limbic striatum consists of the nucleus accumbens and the most ventral putamen and caudate nucleus; it is innervated by the orbitofrontal cortex and anterior cingulate area, hippocampus and amygdala, regions that are included in the revised greater limbic lobe (Heimer 2003).

Patch/striosome and matrix compartments

The dorsal striatum is further divided into patch/striosome and matrix compartments. This compartmentalization was originally defined by the distribution of µ-opioid receptors and acetylcholinesterase activity. Patches/striosomes are rich in µ-opioid receptors (Herkenham & Pert 1981), whereas the matrix is rich in acetylcholinesterase (Graybiel & Ragsdale Jr. 1978). Several other markers also show differential distribution in the compartments. For example, tyrosine hydroxylase, dopamine transporter, dopamine D2 receptor and enkephalin are “confined” to the matrix while dopamine D1 receptor, dynorphin and substance P are predominately localized to the patches/striosomes (see Graybiel 1990). Based on the glutamatergic innervation, the patch/striosome compartment is considered limbic-related and the matrix sensorimotor-related. The patches/striosomes receives glutamatergic projections from amygdala and hippocampus as well as from agranular (deep) cortical layers that are most abundant in the prefrontal and limbic corticies, whereas the matrix is innervated by supragranular (upper) cortical layers predominately from motor and sensory corticies (see Fig. 2; Gerfen 1989).
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Figure 2. A schematic drawing of the functional and compartmental organization of the primate and rodent striatum and their differentiated cortical innervation. The gradient represents the functional subregions of the striatum, white is limbic, gray is associative, and black is motor. The limbic striatum receives innervation from the greater limbic lobe (including orbitofrontal cortex, anterior cingulate area, hippocampus, and amygdala; Heimer 2003, personal communication), the associative from prefrontal corticies, and the motor from the motor cortecies. The cortical innervation to the patch/striosome and matrix compartments differ in layer origin. The patches/striosomes (dotted areas) receive innervation from agranular (deep) layers that are most abundant in prefrontal and limbic corticies, whereas the matrix (surrounding areas) receives innervation from granular (upper) layers that are most dense in sensory and motor corticies.

The origin of the dopamine projections to the compartments also seems to differ (see Joel & Weiner 2000; Prensa & Parent 2001). In rats it has been shown that the patch/striosome compartment receives dopamine innervation predominately from ventral cell populations in the substantia nigra compacta, whereas the matrix receives dopamine innervation from dorsal cell populations. The primate dopamine projections to the patch/striosome and matrix compartments are not well defined.

Connections of the Basal Ganglia

Schematic drawings of the proposed connectivity in the dorsal striatum, nucleus accumbens core, and nucleus accumbens shell are presented in Figure 3. As already described, the striatum receives glutamatergic input mainly from the neo- and allocortex that is modulated by dopaminergic input from the midbrain. In addition, the striatum is innervated by glutamatergic terminals from the thalamus (mainly the intralaminar nuclei), as well as by norepinephrinergic and serotonergic terminals from locus coeruleus and raphe nucleus, respectively (Heimer et al. 1985).
Figure 3. Schematic drawings of the proposed connectivity in the rat caudate-putamen (CAUD-PUT), nucleus accumbens core (ACC-Core), and nucleus accumbens shell (ACC-Shell). There are three major output pathways from the caudate-putamen: the direct pathway expressing dopamine D1 receptors and dynorphin (D1/DYN), the indirect pathway expressing dopamine D2 receptors and enkephalin (D1/ENK), and the patch/striosome pathway (PATCH) expressing predominately D1/DYN and but also D2/ENK. Note that the nucleus accumbens core has similar striatopallidal connectivity as the caudate putamen (but no patch/striosome and matrix organization), whereas the nucleus accumbens shell has more limbic related target regions, such as VTA, mediodorsal (MD) thalamus, and the non-basal ganglia regions extended amygdala (Ext Amy), lateral hypothalamus (Lat Hyp), and brainstem nuclei. EP, entopeduncular nucleus; GP, globus pallidum; SNc, substantia nigra compacta; SNr, substantia nigra reticulata; STN, subthalamic nucleus; VA-VL, ventral anterior, ventral lateral; VPl, lateral ventral pallidum; VPm, medial ventral pallidum; VTA, ventral tegmental area.

There are three major output pathways from the dorsal striatum: the direct, indirect, and patch/striosome pathways (see Graybiel & Penney 1999). Neurons in the direct pathway predominately express D1 receptors and the neuropeptides dynorphin and substance P (Gerfen et al. 1990b; Le Moine et al. 1991) and they project to the internal globus pallidum (entopeduncular nucleus in subprimates) or substantia nigra reticulata. Neurons in the indirect pathway mainly express D2 receptors and the neuropeptide enkephalin (Gerfen et al. 1990a; Huang et al. 1992), and they project to the external globus pallidum and subsequently to the subthalamic nucleus. The subthalamic nuclei projects back to the internal globus pallidum or substantia nigra reticulata. The internal globus pallidum and substantia nigra reticulata projects to the ventral anterior thalamus that primarily innervates the supplementary motor cortex. The direct and indirect pathways originate in the matrix compartment, whereas a separate output pathway arise in the patch/striosome compartment. This third output pathway from the dorsal striatum projects to the substantia nigra compacta. The patch/striosome compartment contains both D1 and D2 receptor cell populations, but predominately D1.
In the ventral striatum, the nucleus accumbens core has similar striatopallidal connectivity as the dorsal striatum (Zahm & Brog 1992a; Kalivas et al. 1993; Lu et al. 1998). The “direct” pathway (D1/dynorphin) projects to the substantia nigra compacta, whereas the “indirect” pathway (D2/enkephalin) projects to the lateral ventral pallidum and subsequently to the VTA or subthalamic nucleus where it enters the same pathway as the dorsal circuitry. In contrast the nucleus accumbens shell has more limbic related target regions. The shell also has a direct (D1/dynorphin) projection pathway, but to the VTA (Zahm & Brog 1992a; Kalivas et al. 1993; Lu et al. 1998). There are also some D1 projecting neurons to the ventral pallidum. The “indirect” pathway (D2/enkephalin) projects to the medial ventral pallidum that innervates both VTA and the mediodorsal thalamus. The mediodorsal thalamus projects to prefrontal corticies that in turn innervates the associative striatum. In addition, the nucleus accumbens shell is interconnected with the extended amygdala (de Olmos & Heimer 1999) and projects to the lateral hypothalamus and brain stem nuclei. In addition to D1 and D2 receptors, the ventral striatum expresses D3 receptors, in particular the nucleus accumbens shell; the dorsal striatum show limited expression (Bouthenet et al. 1991). The D3 receptors are coexpressed with dynorphin but not with enkephalin; presumably some dynorphinergic cell populations express both D1 and D3 receptors (Curran & Watson 1995).

Ventral striatonigral projections can also influence dorsal striatonigral dopamine projections via reveribrating ascending loops suggested by Haber et al (2000). According to this anatomical arrangement, the shell will influence the core, the core will influence the associative striatum, and the associative striatum will influence the motor striatum. Consequently, the reward-related motivational behaviors suggested to be induced by the nucleus accumbens are linked to motor outcomes via these striatonigrostrial pathways in addition to the activation of the mediodorsal thalamus and subsequent cortical stimulation to associative striatum.

1.4.2 Neurochemical substrates

1.4.2.1 Neuropeptides

Neuropeptides are important neuroactive substances that modulate neurotransmission (see Strand 1999). Similar to classical neurotransmitters (i.e., glutamate, GABA, acetylcholine, biogenic amines) neuropeptides are released in a calcium dependent fashion following nerve stimulation. In contrast to other transmitters, neuropeptides are synthesized as prepropeptides that are processed to functional peptide fragments. Neuropeptides are cotransmitters to fast-acting classical transmitters, but the neuropeptide transmission requires greater stimulation (burst firing or high frequency) in order to be initiated, is slow-acting, and has no high affinity termination mechanism (i.e. reuptake). The slow-acting transmission is due to stimulation of metabotrophic G-coupled receptors that modulate the transmission signal via second messenger systems, but not via ion channels (fast-acting). Instead of high affinity termination, extracellular neuropeptides are degraded by peptidases. In addition, the long-lasting action of neuropeptide transmission depends on high receptor affinity and consequently the termination of the transmission is very slow.
Neuropeptides are the most diverse and largest proportion of chemical substances acting as neurotransmitters/neuromodulators. At present, there are more than 100 known active neuropeptides in the brain. Many neuropeptides have been implicated in the pathology of psychiatric disorders, e.g., the opioid peptides (see below), substance P, neurotensin, galanin, neuropeptide Y, cholecystokinin, corticotropin releasing factor (CRF) and oxytocin (Gulya 1990; Lieberman & Koreen 1993; Herpfer & Lieb 2003).

**CART**

One of the most recently identified neuropeptides is cocaine and amphetamine regulated transcript (CART) that was discovered in the rat brain after psychostimulant administration using the technique differential display PCR. The transcript showed enhanced expression in response to acute cocaine or amphetamine administration (Douglass et al. 1995). CART was specifically increased in the striatum, but not in the hippocampus, following either psychostimulant injection. However, the first identification of a CART peptide fragment was made over a decade earlier in extracts of hypothalamus (Spiess et al. 1981). In fact the highest level of CART mRNA in the rat brain is found in the hypothalamus where it represents the third most abundant mRNA expressed in this region (Gautvik et al. 1996).

Following subsequent isolation and characterization of the rat CART mRNA, it was found to encode two protein products (due to alternate splicing): short CART (116 amino acids) and long CART (129 amino acids). In humans, only the short form has been found. The prepropeptide contains a hydrophobic leader sequence (27 amino acids) that indicates secretion (Douglass & Daoud 1996; Adams et al. 1999), and pairs of basic amino acids suggesting post-translational processing into smaller peptides (Thim et al. 1999). Several CART peptide fragments have been isolated from different brain regions, pituitary gland, gut and adrenal gland (Kuhar & Yoho 1999). Furthermore, CART immunoreactivity is localized to large dense core vesicles in nerve terminals (Smith et al. 1997). Taken together, there is accumulated evidence that CART is a neurotransmitter.

In the rat striatum, CART mRNA expression is confined to the nucleus accumbens (Couceyro et al. 1997). There is evidence that accumbal CART is coexpressed with dynorphin-containing projecting neurons to the VTA (Dallvechia-Adams et al. 2002). In addition to the ventral striatum and hypothalamus, high CART mRNA levels were also detected in the induseum griseum, dentate gyrus of the hippocampus, amygdala, medial septum, bed nucleus of the stria terminalis, Edinger-Westphal nucleus, and primary somatosensory cortex of the rat brain (Douglass et al. 1995; Couceyro et al. 1997). The anatomical localization of the CART mRNA and peptide immunoreactivity is largely overlapping (Koylu et al. 1998; Vrang et al. 1999). At the initiation of this thesis project, limited information was available about the CART mRNA expression distribution in the human brain, although northern blot analyses revealed expression in the hypothalamus, frontal cortex, midbrain, hippocampus, and motor cortex (Douglass & Daoud 1996).
Although the CART receptor/s have yet to be identified, there are apparent physiological functions of the peptides. CART peptides are involved in behaviors related to feeding, anxiety, stress, locomotor activity and reward (for review see Kuhar 2002). These functions will be discussed in later sections of this thesis.

Opioid peptides

Long before the discovery of endogenous opioid peptides, man used opium for its analgesic, sedative, and euphoric effects. Opium was obtained from the fruit capsules of the opium poppy. The term opiate characterizes the centrally active substances in opium. Morphine is the most potent natural opiate and is highly addictive. Heroin is a synthetic opiate that rapidly enters the brain and hence has extremely high abuse potential.

Binding sites for opiates in the central nervous system were identified in the early 1970s by several independent research laboratories and were termed opioid receptors (Pert & Snyder 1973; Simon et al. 1973; Terenius 1973). The endogenous ligands to these receptors, the opioid peptides, were discovered shortly thereafter (Hughes et al. 1975; Terenius & Wahlstrom 1975; Lord et al. 1977; Goldstein et al. 1979). The classical opioid peptides are enkephalins, β-endorphins and dynorphins. But in the 1990s new opioid peptides were discovered, the endomorphins (Zadina et al. 1997) and the dynorphin-like nociceptin (Meunier et al. 1995; Reinscheid et al. 1995). All opioid peptides, except for nociceptin, have the same N-terminal sequence Tyr-Gly-Gly-Phe-Leu/Met. This sequence is crucial for binding to the opioid receptors.

There are three subtypes of opioid receptors, mu (µ), kappa (κ) and delta (δ) (Martin et al. 1976; Lord et al. 1977). In general all these receptors are G-protein receptors inhibitory coupled to adenylate cyclase. The opioid peptides bind with different affinity to these receptors (see Mansour et al. 1995). The enkephalins have highest affinity to the delta receptor, but binds also to mu receptors. β-endorphin binds with similar affinity to both mu and delta receptors. The dynorphins are the only opioid with high affinity for the kappa receptor (Chavkin et al. 1982). The newly discovered endomorphins are highly selective mu receptor ligands (Zadina et al. 1997). The rewarding effects of opioids are mediated through mu and delta receptors. In contrast, selective kappa receptor agonists produce dysphoria and place aversion (Pfeiffer et al. 1986; Bals-Kubik et al. 1993).

Dynorphin

The mu and delta receptor systems have received most attention in the research of substance dependence and mood disorders because of their mediation of reward. However, during the last decade the kappa/dynorphin system and mediation of negative effects have been implicated as a neuroadaptive substrate for these disorders. Prodynorphin is the precursor protein (propeptide) of the dynorphins, (Kakidani et al. 1982) and gives rise to several biologically active peptide fragments, dynorphin A,
Dynorphin B, dynorphin B29, dynorphin 32, α-neoendorphin and β-neoendorphin. Dynorphin A and dynorphin B can be enzymatically degraded to Leu-enkephalin resulting in a change in receptor affinity from kappa to delta (Nyberg & Silberring 1990). The dynorphins are predominately found in the hypothalamic pituitary axis, striatum, and hippocampus. In the human brain the prodynorphin gene, coding for preprodynorphin, is predominately expressed in limbic regions including medial prefrontal cortex, amygdala, dentate gyrus and striatum (Hurd 1996). The nucleus accumbens and patch compartment of the caudate nucleus and putamen expressed high levels of the prodynorphin transcript in particular (Hurd & Herkenham 1995). A similar prodynorphin mRNA distribution pattern is found in the rat brain. The dynorphin and its kappa receptor have some overlap in their mRNA distribution suggestive of local opioid circuits, for example the striatum, central amygdala, olfactory tubercle, paraventricular nucleus, and the locus coeruleus (Mansour et al. 1994). The patch matrix organization is not as evident for the kappa receptor expression as compared to dynorphin. Conversely the VTA, substantia nigra reticulata, and compacta express kappa but not prodynorphin mRNA.

1.4.2.2 Dopamine

Dopamine was first identified as a neurotransmitter by Carlsson et al in the late 1950s (Carlsson et al. 1957; Carlsson 1959). Dopamine is involved in a variety of functions relevant to motor control, emotional regulation, reward, motivation and cognition. As a catecholamine, it is synthesized from the amino acid tyrosine by the rate-limiting enzyme tyrosine hydroxylase (see Cooper et al. 1996). The intermediate product L-dihydroxyphenylalanin (L-DOPA) has been successfully used as treatment therapy for Parkinson patients who are characterized by low dopamine levels. L-DOPA is rapidly converted into dopamine and the transmitter product is concentrated in synaptic vesicles and released in a calcium dependent manner following nerve impulse stimulation. The dopamine transmission is mainly terminated by reuptake into the nerve terminals via the dopamine transporter where cocaine acts. Cocaine binds to the dopamine transporter (Heikkila et al. 1975) and blocks the reuptake resulting in excessive extracellular dopamine levels (Hurd & Ungerstedt 1989; Pettit et al. 1990) and a prolonged dopamine transmission.

Dopamine receptors

The various effects of dopamine are mediated through two classes of dopamine receptors, the D1-like (D1 and D5) and D2-like (D2, D3, D4) receptor families (see Seeman & Van Tol 1994; Jaber et al. 1996). The receptors are G-coupled transmembrane proteins that are positively and negatively coupled to adenylate cyclase. Stimulation of D1-like receptors results in increased cyclic adenosine 3’, 5’ monophosphate (cAMP) levels, whereas D2 receptor stimulation reduces cAMP. Both classes of receptors are found post-synaptically, but only D2-like receptors are presynaptically expressed as autoreceptors.

The distribution of D1 and D2 receptors are widespread and largely overlapping, but colocalization in the same neuron is rare according to in situ hybridization studies (Le
Moine & Bloch 1995). Both receptors are found in the caudate-putamen, nucleus accumbens, olfactory tubercle, amygdala, septal area, hypothalamus, and cerebral cortex. The D2 receptors are also expressed by dopaminergic neurons in the substantia nigra compacta, VTA and hypothalamus. Of the other receptor subtypes, the D3 receptor has the most limbic distribution pattern and is expressed in the nucleus accumbens shell, hippocampus, septum and temporal corticies (Bouthenet et al. 1991).

1.4.3 Implications

1.4.3.1 Dopamine reward circuitry

Cocaine acts by blocking the monoamine transporters, dopamine, norepinephrine and serotonin (Kuhar et al. 1991), resulting in increased extracellular levels of these transmitters. The prolonged transmission leads to excessive stimulation of receptors in the monoaminergic target regions. The dopamine system has been most implicated in the stimulatory and reinforcing action of cocaine. Early studies reported that activation of the mesocorticoliclimbic dopamine pathway are rewarding. Olds and Milner (1954) first demonstrated that stimulation of the medial fore brain bundle (including all monoamine projections) induced intracranial self-stimulation (ICSS; Olds & Millner 1954). In the ICSS paradigm, rats are allowed to electrically stimulate specific brain regions by pressing a lever. Reward is demonstrated if lever pressing is continued; this action can often occur to the exclusion of other behaviors. Dopamine was found to be the critical substrate for the ICSS (Fouriezos et al. 1978). In 1987 it was suggested by Wise and Bozarth that the mesocorticoliclimbic dopamine pathway is a common site of action by addictive drugs (Wise & Bozarth 1987). Since then, there is accumulated evidence of the involvement of this pathway in reinforcement, motivation and mood (see Koob 1996; Wise 1996; Schultz 1998). Consequently the mesocorticoliclimbic pathway is considered the main brain reward circuitry.

The mesocorticoliimbic target region that has received most attention in the mediation of reward is the nucleus accumbens. In general most drugs of abuse initially increase extracellular dopamine levels in this region (Di Chiara & Imperato 1988; Koob 1992). Furthermore, maintenance of cocaine self-administration (Roberts et al. 1980; Pettit et al. 1984), as well as the expression of sensitization (Robinson & Berridge 1993), is dependent on nucleus accumbens dopamine transmission. The role of nucleus accumbens dopamine overflow in reward is primarily based on rodent studies, but support of its significance in positive reinforcement was recently demonstrated also in primates and humans. Bradberry et al (2000) found enhanced cocaine-evoked dopamine overflow in the ventral striatum as compared to the dorsal striatum using in vivo microdialysis in monkeys. Similarly, a human neuroimaging study revealed greater reductions in D2 receptor availability (indicative of enhanced dopamine release) in the nucleus accumbens as compared to associative the striatum after amphetamine administration (Martinez et al. 2003). Moreover, the level of amphetamine-evoked euphoria was found to be associated with the magnitude of change in apparent dopamine levels. The reward circuitry is not only activated by addictive drugs but also by natural rewards such as food, water, sex and excessive physical training. There is evidence that these rewards are mediated by endogenous opioid peptides, (Janal et al.
1984; Tanda & Di Chiara 1998; Lett et al. 2001) that interacts with the mesocorticolimbic dopamine system (see section 1.4.3.3).

1.4.3.2 Dopamine hypothesis in depression
The dopamine hypothesis from the 1970s suggested a reduced dopamine tone in a depressed state (Randrup & Braestrup 1977). However, considering the diverse symptoms of depression, a one-transmitter hypothesis for the pathophysiology underlying depression is unlikely. Indeed there is evidence regarding involvement of a variety of neurotransmitter systems such as serotonin, norepinephrine, acetylcholine, GABA, corticotropin releasing factor, neuropeptide Y, somatostatin, substance P and opioid peptides (for review see Markou et al. 1998). In particular serotonin and norepinephrine are thought to be critical transmitter systems considering the useful antidepressant effect when targeting these systems (Blier & de Montigny 1998; Brunello et al. 2002). Nevertheless, dopamine is still suggested to be involved in the pathophysiology of depression. Based on the role of mesocorticolimbic dopamine in reward and motivation, this system is proposed to underlie anhedonia and loss of motivation that are core symptoms of depression.

Originally, the dopamine hypothesis was based on the decreased cerebrospinal fluid (CSF) levels of the dopamine metabolite homovanillic acid (HVA) reported in depressed patients (Goodwin et al. 1973; Asberg et al. 1984). It was supported by the higher incidence of depression in Parkinson’s disease (dopamine deficiency) than in patients with other equally disabling illnesses (Cummings 1985; Mayeux 1990). In addition, preclinical studies revealed chronic antidepressant treatment, i.e. tricyclics, electroconvulsive shock or atypical antidepressants, to increase striatal extracellular dopamine levels (Nomikos et al. 1991) and to enhance responsivity to dopamine agonists (Maj et al. 1987; Brown et al. 1991). Recent neuroimaging studies provide further evidence for the involvement of dopamine in depression. Reduced endogenous dopamine concentrations assessed by D2 availability have been reported (D'Haeenen H & Bossuyt 1994; Shah et al. 1997), but no difference has also been found (Parsey et al. 2001). In agreement, there are reduced L-DOPA levels in the caudate nucleus of depressed subjects (Martinot et al. 2001) and dopamine turnover has been found to correlate to the clinical status of depressed patients (Lambert et al. 2000).

1.4.3.3 Opioid and dopamine interactions
The dopamine and opioid system have tight anatomical interactions and thus functional interactions. Endogenous opioids can modulate basal activity of dopaminergic neurons in the mesocorticolimbic pathway. Stimulation of mu receptors located on GABAergic interneurons in the VTA (Johnson & North 1992), results in disinhibition of dopamine neurons which leads to increased firing and subsequent elevated dopamine release in e.g., the nucleus accumbens (Spanagel et al. 1992). In contrast, antagonism of mu and delta receptors in the VTA reduce accumbal dopamine overflow (Spanagel et al. 1992; Devine et al. 1993). Hence it is through stimulation of mu receptors in the VTA that morphine activates the mesocorticolimbic dopamine reward circuitry. Similarly, alcohol (Gianoulakis & de Waele 1994) and natural reinforcers (Hoffmann et al. 1990)
are suggested to cause release of β-endorphin, the endogenous ligand for the mu receptor. The kappa receptor has the opposite effect on dopamine transmission. Stimulation of kappa receptors in the nucleus accumbens reduces, whereas kappa antagonists increases dopamine overflow (Spanagel et al. 1992). kappa agonist administered into VTA has no effect on accumbal dopamine release, however dopamine overflow in the dorsal striatum are reduced by kappa stimulation in the substantia nigra (Reid et al. 1988). The reduced dopamine overflow following kappa receptor is not only associated with a decrease in dopamine release but also with an increase in dopamine uptake (Thompson et al. 2000).

Dopamine can also modulate the activity of opioid peptides expressed in the striatal projection neurons. Stimulation of D2 receptors regulates the activity of the opioid peptide enkephalin in the striatopallidal pathway. Dopamine depletion by the neurotoxin 6-hydorxidopamine results in increased striatal enkephalin expression, an effect that can be reversed by selective D2 agonist administration (see Steiner & Gerfen 1998). Conversely stimulation of D1 receptors in the striatonigral pathway, leads to enhanced prodynorphin mRNA transcription (Gerfen et al. 1990b) and increased nigral dynorphin levels (Nylander & Terenius 1987; You et al. 1994). Dopamine denervation leads to reduced striatal dynorphin expression, which can be reversed by selective D1 receptor agonist administration (see Steiner & Gerfen 1998). Consistently, transgenic mice lacking the D1 or D2 receptors display reduced dynorphin and enhanced enkephalin expression, respectively (Xu et al. 1994; Baik et al. 1995).

Similar to D1 agonists, administration of the indirect dopamine agonist cocaine, results in enhanced dynorphin peptide in the striatum, nucleus accumbens, and substantia nigra (Sivam 1989; Smiley et al. 1990). In addition, striatal prodynorphin mRNA levels are upregulated following cocaine administration in the rat (Hurd et al. 1992; Daunais et al. 1993; Spangler et al. 1993) in a D1 dependent manner (Spangler et al. 1996b). The up-regulation of prodynorphin mRNA is also observed in the dorsal striatum of human cocaine addicts (Hurd & Herkenham 1993). Not only psychostimulants but also morphine, ethanol, and excessive running increase prodynorphin mRNA expression and peptide tissue levels in the striatum (Przewlocka et al. 1997; Turchan et al. 1997; Lindholm et al. 2000; Werme et al. 2000). The cocaine-evoked modulation of dynorphin is paralleled by increased striatal kappa receptor densities in human postmortem reports (Hurd & Herkenham 1993; Staley et al. 1997) and in rat studies after long-term administration (Unterwald et al. 1994b; Collins et al. 2002).

**Functional considerations**

Based on the hypothesis that drugs that inhibits dopamine release in the nucleus accumbens will suppress drug-seeking behavior, a number of studies have been conducted to examine kappa receptor agonists on the effects of addictive drugs. Both neurochemical and behavioral effects of psychostimulants, opiates, and alcohol can be attenuated by kappa receptor stimulation. Pretreatment with systemic kappa agonists attenuates elevation of dopamine overflow following cocaine, amphetamine, morphine, and heroin administration (Maisonneuve et al. 1994b; Shippenberg et al. 1996; Xi et al. 1998; Staley et al. 1997; Unterwald et al. 1994b).
Similarly kappa agonists can block the cocaine-induced development of behavioral sensitization. Both sensitization to the locomotor activating effect (Heidbreder et al. 1993; Heidbreder et al. 1995) and to the conditioned reinforcing effect (Shippenberg & Rea 1997) is attenuated by stimulation of the kappa receptor. Kappa agonists have also proven effective in the attenuation of self-administration behavior. Maintenance (Glick et al. 1995; Schenk et al. 1999; Schenk et al. 2000), but not acquisition (Schenk et al. 2001), of low dose cocaine self-administration can be attenuated. Likewise, kappa mediated disruption of cocaine self-administration has been reported in the rhesus monkey (Negus et al. 1997; Mello & Negus 1998). Considering the potential treatment of cocaine addiction by kappa agonists, it is of particular interest that the reinstatement of cocaine-seeking behavior produced by priming injections of cocaine can be inhibited by kappa stimulation (Schenk et al. 1999). Not only cocaine self-administration behavior is blocked by kappa receptor activation, also heroin, morphine, and alcohol self-administration (Glick et al. 1995; Xi et al. 1998; Lindholm et al. 2001; but see Holter et al. 2000). However, the kappa agonists’ enadoline and butrophanol had no effect on cocaine self-administration in humans, although reduced ratings of feeling “high” were reported (Walsh et al. 2001).

Further evidence of reward modulation by dynorphin has been demonstrated in rats with overexpression of the transcription factor cAMP response element binding protein (CREB) in the nucleus accumbens. One target gene of the CREB transcription factor is prodynorphin. Overexpression of CREB resulted in decreased rewarding effects of cocaine in the conditioned place preference paradigm, which could be blocked by a kappa receptor antagonist (Carlezon et al. 1998).

An increased dynorphin tone has been suggested to underlie a negative mood state. Dynorphin-like agents produce aversive effects in experimental animals (Mucha & Herz 1985; Shippenberg & Elmer 1998) and induce dysphoria in humans (Pfeiffer et al. 1986). Considering the striatal anatomical connectivity of the dynorphin system, the aversive effects of kappa stimulation are likely mediated by kappa receptors in the nucleus accumbens, but possible also via the patch/striosome compartment. Kappa receptor stimulation in the VTA or nucleus accumbens produces conditioned place aversion (Bals-Kubik et al. 1993). In agreement, enhanced dynorphinergic activity in the nucleus accumbens has been demonstrated in association with dysphoria (Pliakas et al. 2001; Newton et al. 2002). Accumbal overexpression of the transcription factor CREB, which regulates dynorphin expression, was found to increase immobility in the forced swim test, indicative of a negative mood state since standard antidepressants decrease immobility. This effect was blocked by the kappa opioid receptor antagonist nor-binaltorphimine, suggesting that CREB-mediated induction of dynorphin indeed contributed to the increased immobility (Pliakas et al. 2001). The antidepressant effect of kappa antagonist was further supported in the forced swim test and learned helplessness (Newton et al. 2002; Mague et al. 2003). Moreover, reduced accumbal prodynorphin mRNA which was down-regulated by local injections of a mutant of CREB was associated with an antidepressant–like effect in the learned helplessness
model (Newton et al. 2002). In humans, elevated prodynorphin mRNA levels has been detected in the limbic-related patch/striosome compartment of suicide subjects (Hurd et al. 1997).

A role in comorbidity

There is accumulated evidence that the dopamine system is impaired in both cocaine dependence and depression. Reduced dopamine transmission is suggested in depressed individuals and during early cocaine abstinence (Parsons et al. 1991; Chefer & Shippenberg 2002). The hypodopaminergic function possibly mediates anhedonia and might be a driving force for the continuation of cocaine intake. The dynorphin system can mediate a hypodopaminergic state in the response to cocaine through counteradaptive processes in the development of dependence. Dynorphin induce dysphoria and produce aversive effects, properties associated with a negative mood. It is therefore possible that elevated DYN activity, and the negative mood state it produces, is a core feature of depression and drug dependence. Consequently, the DYN system might be a potential target for pharmacological interventions of these disorders.

1.4.3.4 CART and dopamine interactions

Injections of the CART peptide into the VTA results in increased locomotor activity and conditioned place preference (Kimmel et al. 2000). This effect was blocked by the D2 receptor antagonist haloperidol, suggesting the CART action to be mediated by dopamine. However, sensitization or tolerance of CART-evoked locomotor activity was not induced by repeated injections of the peptide, or after a challenge psychostimulant injection. Nevertheless, the findings reveal that the CART peptide has rewarding and locomotor stimulating effects similar to psychostimulants when administered into the VTA.

CART immunoreactivity is found in GABAergic terminals in the VTA. Thirty % of the CART terminals in the VTA target dopamine dendrites whereas the majority targets presumably GABAergic interneurons. Hence, the CART peptide have two possible sites of action to mediate its effects on dopamine activity: 1, direct on the dopamine neuron or 2, indirect via disinhibition of GABAergic interneurons. Retrograde tracing studies from VTA have revealed that the majority of CART neurons originate in the hypothalamus (lateral and perifornical areas) and to a lesser extent in the nucleus accumbens (Dallvechia-Adams et al. 2002). Both these regions are involved in the rewarding effects of drugs of abuse: nucleus accumbens as a mesolimbic target region of the reward circuitry and the hypothalamus as a target for opioid actions. Morphine and enkephalin are self-administered (Olds & Williams 1980) into the lateral hypothalamus and induce conditioned place preference after local administration in this region (van der Kooy et al. 1982; David & Cazala 1994). However not all studies are in agreement (Bals-Kubik et al. 1993; Olmstead & Franklin 1997). Taken together CART activity in the VTA can be regulated by two possible projection pathways, further studies are required to elucidate the specific contribution by each pathway.
In addition to the psychostimulant-like effect of CART in reward and locomotion, a direct role of the peptide in anxiety-like behavior has also been demonstrated. Intracerebroventricular (icv) administration of the CART peptide results in a dose-dependent anxiogenic effect on the plus-maze (Kask et al. 2000; Chaki et al. 2003). This effect may also mirror a psychostimulant response since anxiogenic responses are well documented after cocaine or amphetamine administration (Pellow et al. 1985; Rogerio & Takahashi 1992; Yang et al. 1992; DeVries & Pert 1998; Paine et al. 2002).
2 AIMS OF THE STUDY

The overall aim of the project was to add knowledge to the neurobiology underlying cocaine dependence. The main focus was on postsynaptic neuroadaptations of dopamine related striatal neuropeptides at different phases of the psychostimulant abuse cycle. In addition, behavioral disturbances in relation to the comorbidity between cocaine and depression were investigated.

The Specific Aims were to:

- Characterize the anatomical distribution pattern of neuropeptide CART mRNA expression in the human brain and evaluate its possible functional relevance to human cocaine abuse.

- Examine the putative cocaine-regulated expression of the neuropeptide CART mRNA in limbic related brain areas of the rat.

- Determine the temporal activation of striatal prodynorphin mRNA expression at different stages of the cocaine abuse cycle in primates and rats.

- Study the time course of experimental anxiety during cocaine abstinence in the rat.

- Characterize cocaine responsivity in an animal model of depression, including acquisition of cocaine self-administration and in vivo DA transmission in relation to locomotor behavior.
3 MATERIALS AND METHODS

3.1 RAT EXPERIMENTS (PAPER II, V, VI)

3.1.1 Animals (paper II, IV, V, VI)

Albino Sprague-Dawley rats (SD; ALAB, Stockholm, Sweden and B&K Universal, Sollentuna, Sweden) weighing 250-300g (8-9 weeks) at the beginning of the experiments were used in papers II, IV and V. In paper VI, we used the Flinder sensitive line (FSL) and Flinder resistant line (FRL) rats weighing 230-370 g (8-10 weeks) at the beginning of the experiment. The Flinder rat lines came from breeding colonies maintained at the University of North Carolina, USA, and Karolinska Institutet, Sweden. Male rats were used in all rodent studies except paper II where female rats also were investigated. All animals were kept on a 12 h light/dark cycle in a temperature and humidity controlled room with food and water available ad libitum. Animals were treated in accordance with protocols approved by the animal ethical committee of Stockholm (N66/98, N211/94, N191/97).

3.1.2 Surgeries (paper II, VI)

In paper II, female rats were bilaterally ovariectomized by removing of the ovaries from an incision on the back using sodium pentobarbital anesthesia.

In paper VI, for the cocaine self-administration experiments, animals were implanted with a chronically indwelling jugular catheter that exited dorsally between the scapulae under 1% halothane/air anesthesia. The catheters were constructed from a silastic tubing (10 cm, I.D. 0.30 mm x O.D. 0.64 mm, Dow Corning, USA) that was obturated with a bent metal cannula (C313G Plastics One, Virgina, USA) and attached to a piece of mesh. At the end of the surgery, temgesic (0.03 mg/kg Reckit and Colman, Hull, England) and saline (2 ml) were administered (s.c.) for analgesia and to minimize dehydration, respectively. During a seven-day post-surgery period the catheters were flushed daily with heparinized (3U, Lowens lakemedel, Malmo, Sweden) saline to maintain potency and during the first three days antibiotics (10 mg Doctacillin, AstraZeneca, Södertalje, Sweden) to prevent infections.

In paper VI, for sampling of extracellular dopamine levels, animals were stereotactically implanted with a microdialysis guide cannula (shortened by 2mm; CMA/12; CMA/Microdialysis AB, Stockholm, Sweden) aimed at the nucleus accumbens shell under 1% halothane/air anesthesia. The stereotaxic coordinates relative to bregma were: anterior +2.0 mm, lateral 1 mm and ventral 2.0 mm so that subsequent insertion of the probe would reach the depth of 8.0 mm (Paxinos & Watson 1997). The guide cannula was secured with skull screws and dental acrylic cement. At the end of the surgery ampicillin (100 mg/kg, Boehringer Ingelheim, Hellerup, Denmark) and saline (2 ml) were administered (s.c.) to prevent infection and dehydration, respectively.
3.1.3 Cocaine administration (paper II, IV, V, VI)

Cocaine hydrochloride (Apoteket AB, Sweden) was dissolved in physiological saline for administration to the rats.

When cocaine was administered passively, rats received either a single daily intraperitoneal (i.p.) injection (paper IV, V, VI) or daily “binge” i.p. injections (paper II, V). In the “binge” paradigm, rats received 3 injections, 1 h apart. The “binge” drug administration paradigm was developed as an attempt to mimic the intake pattern in human cocaine abusers since repeated administrations are most often seen within one drug session (Branch et al. 1992).

For self-administration (paper VI), the rats were placed in operant chambers (Med Associates Inc., St Albans, Vermont, USA). Each chamber contained a lever and a cue light and was equipped with an infusion pump. Intravenous infusions of cocaine were delivered through a single channel swivel with connector attachments to the implanted catheter. The rats were maintained under a FR-1 schedule of reinforcement where one lever press resulted in an infusion of cocaine as well as illumination of the cue light.

3.1.4 Elevated Plus-maze (paper V)

The elevated plus-maze is, as the name implies, a maze with the shape of a plus. The maze was elevated 50 cm above the floor and consisted of two open and two closed arms (each arm; 50 x 10 cm). Testing was performed under dimmed red light. Prior to testing, subjects were placed in a novel environment (an empty housing cage) for 5 min. Rats were placed on the central area of the maze facing one open arm and were allowed to explore for 5 min. An observer blind to the experimental conditions measured number of arm entries and the time spent on each arm.

3.1.5 Locomotor activity (paper V,VI)

In paper V, locomotor activity was determined by placing subjects in cages equipped with infrared beam detection (Med Associates, St Albans, VT, USA). Interbeam distance was 8.5 cm horizontally and 6.5 cm vertically, activity was recorded for 30 min at 10 min intervals.

In paper VI, locomotion was measured simultaneously with microdialysis sampling in a computer-controlled activity chamber (68 x 68 x 45 cm) that was equipped with infrared beam detection. Interbeam distance was 8.5 cm horizontally and vertically (for details see (Ericson et al. 1991). Due to interference from the microdialysis tubings, the interruptions of the upper photocells were not considered. Activity was recorded at 10 min intervals during novelty for 60 min and following cocaine or saline administration for another 90 min.

3.1.6 Behavioral assessments (paper VI)

Behavior in the novel activity chamber was observed during the initial five min. The number of rearings in the periphery and in the center of the box was counted.
Grooming behavior was scored by indicating 1 for a single grooming event in 60 s, and 2 for continuous grooming during 60s. The maximal score possible was 10.

Cocaine-induced stereotypies were scored using a scale modified from Creese and Iversen (1974) and Kalivas et al. (Kalivas et al. 1988b). The scale ranged from 1-8 as follows: 1, asleep or still; 2, inactive or grooming; 3, active with locomotion, rearing or sniffing; 4, any combination of two locomotion, rearing or sniffing; 5, patterned sniffing over a wide area; 6, patterned sniffing in one location; 7, continuous licking; 8, bizarre diskinetic movement or seizure.

3.1.7 In vivo Microdialysis (paper VI)

Approximately 15 h before microdialysis sampling, a microdialysis probe (CMA/12 14/02; CMA Microdialysis AB) was inserted into the nucleus accumbens via the guide cannula. At the start of the experiment, the probe was continuously perfused (1 µl/min) with a sterile modified cerebrospinal fluid solution (Na⁺ 148 mmol, K⁺ 2.7 mmol, Mg²⁺ 0.85 mmol, Cl⁻ 155 mmol, Ca²⁺ 1.2 mmol, pH 6.0; Apoteket). Dialysate samples were collected every 10 min and were analyzed for dopamine and its metabolites using high performance liquid chromatography (HPLC) coupled to electrochemical detection. A detailed protocol for the dopamine analysis is described in paper VI.

3.2 POSTMORTEM TISSUE HANDLING

3.2.1 Human brain tissue (paper I)

Normal human brains were obtained at autopsy from the Forensic Medicine Department at the Karolinska Institute under guidelines approved by the ethics committee and the Swedish Board of Health and Social Welfare (120-2263/94). Whole hemispheres of human brains were frozen and stored at -85°C until cryosectioned (100 µm-thick) using a heavy duty microtome (LKB2250, LKB, Stockholm, Sweden). Additional human brain specimens were cut into coronal blocks, frozen in isopentane at -40°C and stored at -40°C until cryosectioned (20 µm) using a Jung-Frigocut 2800E cryostat (Leica, Heidelberg, Germany).

3.2.2 Monkey brain tissue (paper III)

Sectioned monkey brain tissue was obtained from the Wake Forest University North Carolina, USA, in collaboration with Professor Linda Porrino and Professor Mike Nader. The specimen originated from male Rhesus monkeys (Macaca mulatta), 6-13 years old. All animal experimental procedures were approved by Animal Care and Use Committee, USA (A00-089).

3.2.3 Rat brain tissue (paper II, IV)

Rats were anesthetized in a CO₂ chamber and decapitated. The brains were quickly removed, frozen in isopentane at -40°C, and stored at -70°C until cryostat sectioning (20 µm; Jung-Frigocut 2800E cryostat).
3.2.4 Tissue preparation

The cryostat-cut brain sections were immediately thaw-mounted onto glass slides (coated with poly-L-lysine), dried at 37°C, and then stored at -20°C. Prior to in situ hybridization, the slides were fixed in 4% paraformaldehyde/PBS (0.9% phosphate buffered saline) for 5 min, rinsed twice in PBS and treated with 0.25% acetic anhydride/0.1 M triethanolamine/0.9% sodium chloride for 10 min, rinsed in 2X standard saline citrate (SSC), dehydrated in graded series of ethanol (70%, 80%, 95%, 100%), delipidated with chloroform for 5 min, rehydrated in ethanol (100%, 95%) and air dried. All solutions were made with autoclaved 0.1% diethylpyrocarbonate treated water.

3.3 IN SITU HYBRIDIZATION (PAPER I, II, III, IV)

3.3.1 Antisense probes

The human CART RNA probe was synthesized from a 796 bp human cDNA (encoding the full-length human CART gene; Douglass & Daoud 1996) inserted into a pGEM-3Z vector (kindly provided by Dr. P. Couceyro).

The rat CART RNA probe was made from a cDNA fragment of the rat CART gene (bp 36 – 827; Douglass et al. 1995), subcloned into a pBluescript SK(-) vector (kindly provided by Dr. P. Couceyro).

A human prodynorphin RNA probe, that was made from a 1.2 kb fragment of the human cDNA encoding most of exon 4 of the prodynorphin gene (courtesy of Dr. Jim Douglas; Horikawa et al. 1983) was used for detection of prodynorphin mRNA expression in the monkey.

The rat prodynorphin RNA probe was made from a cDNA fragment of the rat prodynorphin gene (bp 466-1101; Civelli et al. 1985) that had been subcloned in a pGEM-4Z vector.

Synthetic cDNA oligonucleotide probes of 48 bases in length were used for detection of proenkephalin (bp 388-435; Yoshikawa et al. 1984) and prodynorphin (bp 862-909; Civelli et al. 1985), and the dopamine receptors, D1 (bp 828-876; Monsma et al. 1990) and D2 (bp 28-75; Monsma et al. 1989) were used in paper IV.

Detailed protocols for transcription reactions of the riboprobe labeling using 35S-UTP and oligonucleotide end-labeling using 35S-dATP are described in papers I, II, III, IV.

3.3.2 In situ hybridization

The hybridization buffer consisted of 0.5 mg/ml sheared ss DNA, 250 µg/ml yeast tRNA, 1X Denhardt's solution (solution of 0.2% each, bovine serum albumin, ficoll, polyvinylpyrrolidone), 10% (w/v) dextran sulfate, 4X SSC, and 50% formamide. Before hybridization, the labeled probe was added to the hybridization cocktail in a concentration of 20 x 10^3 cpm/µl. The sections were coverslipped to prevent
evaporation and the hybridization was carried out in a chamber, humidified using filter paper soaked in 4X SSC and 50% formamide, overnight at 55°C. Incubation was followed by RNase A treatment (40 µg/ml) for 30 min at 37°C and subsequent washes in a graded series of SSC solutions (2X SSC, 2 x 5 min; 1X SSC and 0.5X SSC, 10 min; 0.1X SSC, 1 h) containing 1 mM dithiothreitol, all at room temperature except for the 0.1X SSC (53°C). Dehydration was carried out with graded ethanol solutions containing 300 mM ammonium acetate. The slides were then air dried and exposed to β-max Hyperfilm (Amersham, Buckinghamshire, UK) along with 14C standards (American Radiolabeled Chemicals, St Louis, MO, USA).

3.3.3 Image analysis

Optical densities were determined from digitalized images (scanned by ScanMaker III; Microtek Electronics, Düsseldorf, Germany) using a Macintosh-based image analysis software system (IMAGE; Wayne Rasband, NIMH, MD, USA). Using the coexposed standards, optical density was converted to dpm (disintegration per minute)/mg tissue. Different regions of interest were examined from the brain images in conjunction with anatomical atlases (human; Duvernoy 1991; rat; Paxinos & Watson 1997).

3.4 STATISTICAL ANALYSIS

All statistical analyses were carried out using JMP (3.1v, SAS institute Inc, Cary, NC, USA) or Statistica (5.5v, StatSoft Inc, Tulsa, OK, USA) statistical software, except for the mixed model ANOVA where SAS (SAS institute Inc, Cary, NC, USA) was used. The Welch ANOVA was used to determine significant differences for unequal variances (paper III). The non-parametrical ranking test Mann-Whitney was used to analyze the stereotypy scores (paper VI). A mixed-model ANOVA for inhomogeneous variances was used to analyze the dose-response function data (paper VI). In all other experiments statistical significance was determined by using one- or two-way ANOVAs with repeated measures where appropriate (e.g. paper VI). Significant ANOVA results were followed by Tukey-Kramer post-hoc comparison tests.
4 RESULTS AND DISCUSSION

4.1 CART mRNA EXPRESSION

The CART gene transcript was first discovered in the rodent brain where it showed enhanced expression in response to acute cocaine or amphetamine administration (Douglass et al. 1995). A potential role in drug abuse was further implicated by the specific anatomical distribution pattern of the CART system in the rat, where it is confined to limbic and neuroendocrine brain regions (Couceyro et al. 1997; Koylu et al. 1998). Prior to the publication of Paper I, the expression pattern in the human brain had yet to be described. This section will elucidate the anatomical distribution pattern of neuropeptide CART mRNA expression in the human brain and evaluate its possible relevance to human cocaine abuse (paper I). A subsequent examination in the rat of the suggested regulation of CART transcription following acute cocaine administration will also be discussed (paper II).

4.1.1 Anatomical organization of the human brain (paper I)

We studied the expression of the CART gene transcript in the human post-mortem brain using \textit{in situ} hybridization histochemistry. Similar to the rat, the CART mRNA signal in the human brain revealed a specific expression pattern primarily confined to discrete limbic-, sensory- and neuroendocrine-related regions (Fig. 4). However, some species differences to the rat were observed (Fig 5).

\textbf{Figure 5.} Distribution of CART mRNA in coronal sections of the rodent brain. Arc, arcuate nucleus; BNST, bed nucleus of stria terminalis; CeA, central amygdala; Co, nucleus accumbens core; C-P, caudate-putamen; DG, dentate gyrus; F, fundus; IG, induseum griseum; LH, lateral hypothalamus; LSh, lateral nucleus accumbens shell; MeA, medial amygdala; MSh, medial nucleus accumbens shell; PoA, posterior cortical amygdala; PVN, paraventricular nucleus; S, septum; SCx, sensory cortex; T, thalamus
Figure 4. Distribution of CART mRNA expression in the human brain. The images represents whole brain coronal cryosections, hybridized with CART antisense probes, at three rostrocaudal levels (A-C). aCg, anterior cingulate gyrus; Amy, amygdala; AN, anterior thalamic nucleus; cc, corpus callosum; CeA, central amygdala; CN, caudate nucleus; DLPFC, dorsolateral prefrontal cortex; EC, entorhinal cortex; F, frontal lobe; GPe, globus pallidus, external; GPi, globus pallidus, internal; Hipp, hippocampus; I, insula; MeA, medial amygdala; MD, mediodorsal thalamus; NAc, nucleus accumbens; OPFC, orbital prefrontal cortex; P, parietal lobe; PC, parietal cortex; pCg, posterior cingulate gyrus; Pu, putamen; PVN, paraventricular hypothalamus; S, subiculum; SN, substantia nigra; T, temporal lobe; VI, ventral lateral thalamus; ZI, zona incerta.

4.1.1.1 CART mRNA expressing brain regions relevant for cocaine abuse

The anatomical distribution pattern of the human CART mRNA expression was highly localized to regions relevant to cocaine abuse. A schematic overview of these positive CART labeled regions is presented in Figure 6.

Mesocorticolimbic brain regions

The mesocorticolimbic dopamine system is highly implicated in the actions of drugs of abuse as it is considered the major reward pathway. We found most target regions of these dopamine projections to express CART mRNA; the nucleus accumbens, amygdala complex, bed nucleus of stria terminalis, hippocampus, and the orbitofrontal, piriform, and entorhinal corticies.
Figure 6. Schematic illustration of CART-expressing brain regions that are relevant to the actions of cocaine. The mesocorticolimbic pathway (see projection arrows) originates from the dorsal tier dopamine neurons. Dark grey; target regions of the mesocorticolimbic dopamine pathway. Black; non-mesocorticolimbic regions implicated in the actions of cocaine. Striped; other regions with high CART mRNA expression. AcbS, nucleus accumbens shell; AMY, amygdala; A, anterior thalamic nucleus; BNST, bed nucleus of stria terminalis; DLPFC, dorsolateral prefrontal cortex; DR, dorsal raphe; DT, dorsal tier; EC, entorhinal cortex; Hipp, hippocampus; Hyp, hypothalamus; LC, locus coeruleus; MD, mediodorsal thalamus; OPFC, orbital prefrontal cortex; PC, parietal cortex; PCC, posterior cingulate cortex; Pir, piriform cortex

In the nucleus accumbens, the key region of the reward circuitry, we found a positive CART hybridization signal, whereas no labeling was found in the caudate nucleus or the putamen. The expression of the CART mRNA confined to the ventral, but not dorsal, striatum is consistent with an involvement of CART in cocaine abuse. In addition, the CART labeled cells were predominantly localized to the “shell-like” region of the nucleus accumbens. It has been demonstrated that elevated dopamine overflow is more pronounced in the shell as compared to core following the administration of several addictive drugs in rats (Pierce & Kalivas 1995; Pontieri et al. 1995). In fact cocaine is self-administered exclusively into the shell of the nucleus accumbens (Rodd-Henricks et al. 2002). However, it should be noted that the CART mRNA expression levels in the human nucleus accumbens were low; 60% of the subjects showed no detectable CART hybridization signals. However, the analyzed
brain specimens were obtained from normal controls. It remains to be investigated if this low expression is activated in cocaine dependent individuals.

The amygdala complex showed high expression of CART mRNA. The amygdala has been strongly implicated in cocaine craving and conditioning. Imaging studies have shown amygdala activation during cue-induced cocaine craving in humans (Grant et al. 1996; Childress et al. 1999; Bonson et al. 2002). Furthermore, lesions of the basolateral amygdala in the rat was found to block cue-induced reinstatement of cocaine self-administration (Meil & See 1997) and to attenuate acquisition of cocaine self-administration under second order schedules of reinforcement (Whitelaw et al. 1996). In addition, the amygdala is involved in the expression of anxiety, which was found to be induced by icv CART peptide administration (Kask et al. 2000; Chaki et al. 2003).

In addition to the amygdaloid complex, the extended amygdala expressed positive CART mRNA labeling. The bed nucleus of stria terminalis and centromedial amygdala constitutes the extended amygdala, a continuum of neurons that are interconnected with the nucleus accumbens shell (de Olmos & Heimer 1999). The extended amygdala is considered an output channel from the greater limbic lobe to the neuroendocrine and autonomic nervous systems (Heimer 2003) and has recently received attention for its role in drug dependence (McGinty 1999). Similar to the nucleus accumbens, the bed nucleus of stria terminalis responds to the acute administration of a variety of drugs of abuse, i.e., nicotine, morphine, ethanol and cocaine, with increased extracellular dopamine levels (Carboni et al. 2000).

High CART mRNA levels were also found in the dorsolateral prefrontal and orbitofrontal corticies. These frontal lobe cortical regions have been implicated in cocaine addiction: neuroimaging studies report lower glucose metabolism during cocaine intoxication and higher metabolism during craving (for review see Goldstein & Volkow 2002). Furthermore, frontal lobe hypofunction has been demonstrated in recovering cocaine dependent patients (Volkow & Fowler 2000; Franklin et al. 2002). The dorsolateral and orbitofrontal corticies are involved in executive function, decision-making, and impulse control, functions that are impaired in cocaine-addicted individuals.

Ventral striatopallidal system

The CART positive labeling in the nucleus accumbens shell was accompanied by labeling in other regions of the ventral striatopallidal system (see Zahm & Brog 1992a). Many of the glutamatergic input projections to the nucleus accumbens originate in CART-expressing regions such as, orbitofrontal and dorsolateral cortex, amygdala complex and hippocampus. Furthermore, the main output region of the ventral basal ganglia, the ventral pallidum exhibited scattered CART labeling. Intense CART mRNA labeling was also found in the mediiodorsal thalamus. This thalamic nucleus receives innervation from the ventral pallidum and provides a major projection to the prefrontal cortex. The ventral striatopallidal circuitry is generally considered the motivation-based motor executor, controlled by the reward circuitry.
Cocaine effects on striatal dynorphin and CART neuropeptides

Limbic Thalamus

Impairments in emotional processing are features of cocaine dependence. The CART expressing anterior thalamus, in addition to the mediodorsal thalamus, is involved in such processes considering its limbic anatomical connectivity. The anterior thalamus is a part of the Papez’s circuit in which it transfers information from the mammillary bodies to the cingulate cortex (Papez 1937). The anterior cingulate, in addition to the amygdala, was found to be activated during cue-induced cocaine craving (Childress et al. 1999). Similarly, the anterior thalamus was activated in alcoholic subjects after alcohol-specific cue exposure (George et al. 2001).

Hypothalamus

The hypothalamus showed the highest total CART mRNA expression levels of the brain areas studied. Positive labeling was found in nuclei implicated in the control of appetite, e.g., the arcuate, dorsomedial, ventromedial and paraventricular nuclei, indicative of a role for CART in human energy homeostasis. In the rat, CART peptides have been shown to regulate food intake, for example icv injections of the peptide inhibits feeding (Kristensen et al. 1998; Asakawa et al. 2001; Bannon et al. 2001). Therefore it is possible that hypothalamic CART plays a role in the anorexic effects of cocaine. Moreover, there is evidence that CART is also involved in stress, possible via hypothalamic CRF (see Kuhar et al. 2002). It is well documented that cocaine influences the stress response, and it has been suggested that stress reduction may be effective in reducing craving and promoting abstinence in cocaine addicts (see Goeders 2002).

Monoaminergic cell populations

Of the monoaminergic cell populations in the brainstem, the locus coeruleus (norepinephrinergic) expressed highest CART mRNA levels. Scattered labeling was also found in the dorsal raphe (serotonergic), but no positive labeling was detected in the substantia nigra compacta or VTA (dopaminergic). Although the dopamine system has been most implicated in reward and reinforcement, cocaine acts at all monoaminergic transporters; subsequently the norepinephrine and serotonin systems are also important to the actions of cocaine. Furthermore there is a direct link between these neuronal populations and the mesocorticolimbic dopamine systems since the dorsal raphe and locus coeruleus innervates the VTA (Heimer et al. 1985). In addition these systems are highly implicated in the pathophysiology of depression; clinically effective antidepressant pharmacotherapy acts at the norepinephrine and/or serotonin systems (Blier & de Montigny 1998; Brunello et al. 2002).

4.1.1.2 Species differences between the human and rat brain

The hypothalamus and amygdaloid complex showed a good similarity between the human and rat, whereas apparent species differences were found in other regions. Similar to the human, the rat neocortex showed a heterogeneous pattern of CART mRNA expression, positive labeled areas were visible immediately adjacent to areas
with low or absent CART mRNA expression. However, the high CART mRNA expression in the human dorsolateral prefrontal, orbitofrontal, and temporal corticies were not matched in the rat brain. Furthermore, the human cortical laminar distribution pattern of the CART mRNA was predominantly localized to layer II, whereas the rat showed positive cells localized to layer IV. Both layers are granular with corticocortical projections. In the striatum, the confinement of positive CART mRNA labeling to the "shell-like" region of the human nucleus accumbens was not matched in the rat. Rats showed very high expression of the CART mRNA throughout the nucleus accumbens in the shell, core, and rostral pole. Similarly, both human and rat hippocampus expressed the CART transcript, but in different cell populations. In the human hippocampal formation, CART mRNA was primarily localized within the CA region and polymorphic layer of the dentate gyrus which showed no or weak expression in the rat. In contrast, the human subiculum showed very low levels, whereas the rat subiculum and rostral dentate gyrus were intensely labeled. However, the evolutionary "old" hippocampal rudiment induseum griseum was intensely labeled in both the human and rat brain. The greatest species difference was found within the thalamus. In the human brain, high CART mRNA expression was present in various thalamic nuclei: the pulvinar, mediodorsal, anterior, lateral dorsal, ventral posteriomedial and lateral posterior, as well as in lateral geniculate, medial geniculate, and zona incerta; whereas in the rat, CART mRNA is absent from most thalamic nuclei except for the reticular thalamus (which was not labeled in the human), and zona incerta (Douglass et al. 1995; present results).

Based on our limited knowledge of the functional role of CART, it is difficult to account for the marked species difference of the CART mRNA expression. Whether these differences are due to the influence of post-mortem interval, hormonal status, age, or gender in the human subjects has to be further explored.

**4.1.2 Cocaine effects in the rat (paper II)**

The potential role of CART in reward and reinforcement was further implicated by the specific expression of CART in human brain regions relevant to cocaine addiction. It would have been of great interest to study the mRNA expression of CART in human cocaine users, but unfortunately we did not have access to such brain specimens. Instead, in paper II, we investigated the CART mRNA expression in the male and female rat after acute “binge” cocaine administration. The aspects of gender and possible regulation by estradiol were included in this study considering the reported gender differences in the response to psychostimulants. Women report lower ratings, as compared to men, for the subjective effects of psychostimulants in the luteal phase (low estradiol) but similar to men during the follicular phase (high estradiol) (Sofuoglu et al. 1999; White et al. 2002). Furthermore, women experience increased cocaine craving (Robbins et al. 1999) and more impulsive relapses (McKay et al. 1996) than men.

In paper II, male and female rats received three injections, 1 h apart, of cocaine (15 mg/kg) or saline. The female rats underwent bilateral ovariectomy a week prior to the
cocaine treatment. In addition the females had received a single injection of 17β-
estriadiol or vehicle 24 h before the cocaine administration.

### 4.1.2.1 Basal gender differences

We found a significant effect of gender in the expression of CART mRNA in the shell-
region of the nucleus accumbens. The male rat expressed higher levels of the transcript
as compared to ovariectomized female rats (120-130%, p < 0.05). Gender differences
have previously been detected in the striatum, with focus on the dopamine system.
There are differences in dopamine receptor densities (Rivest et al. 1995; Andersen et al.
1997) and responsivity to dopamine release (Sershen et al. 1998) between the sexes. It
is possible that the gender difference currently observed in the CART mRNA
expression is connected to the dopaminergic differences since the CART peptide is
localized to dendrites in close proximity to dopamine terminals in the accumbens
(Smith et al. 1999; Dallvechia-Adams et al. 2002). Furthermore, it is interesting that the
gender difference was found in the shell compartment that is the most limbic associated
area of the accumbens interconnected with the extended amygdala (Zahm & Brog
1992b). Impairments of the limbic system are relevant in psychiatric disorders such as
depression, and depression is more common among women than men (Paykel 1991;
Lehtinen & Joukamaa 1994). Furthermore, it is possible that the CART system has a
role in the pathology of mood disorders considering the anxiogenic effects of CART
peptides in rodents (Kask et al. 2000; Chaki et al. 2003).

### 4.1.2.2 Regulation by estradiol

Despite noted gender differences, we found no evidence that the CART transcription is
acutely regulated by estrogen since a single estradiol treatment did not affect the
expression of CART mRNA in the brain regions studied. There might be an interaction
between cocaine and the estradiol treatment since activation of the transcript was only
observed in the non-estradiol treated females after the cocaine injections (see next
section).

### 4.1.2.3 Gender specific effects of acute cocaine

There were also gender differences in the response to cocaine. The female vehicle-
(not estradiol-) treated rats exhibited higher CART mRNA levels in the medial tip of the
accumbens shell after the cocaine injections, whereas the male rats expressed elevated
levels in the central amygdala (Fig 7). These results do not confirm the original finding
that the striatal CART mRNA expression is acutely regulated by administration of
psychostimulant drugs in the male rat (Douglass et al. 1995). Lack of activation of the
CART gene in our study could be due to usage of different cocaine administration
paradigms and different mRNA detection techniques as compared to the original report.
However, consistent with our finding, Vrang et al (2002) found no activation of the
CART transcript in the male nucleus accumbens following amphetamine
administration. Nevertheless, we did find an activation of the CART transcript in the
male central amygdala and in the accumbens of non-estradiol treated females, which
Figure 7. The effect of acute binge cocaine administration on the CART mRNA expression in the medial nucleus accumbens shell (A) and the central amygdala (B) of male and ovariectomized 17β-estradiol and vehicle treated female rats. Rats received three injections of either cocaine (15 mg/kg) or saline at one-hour intervals. Fem-Veh, ovariectomized vehicle treated female rats; Fem-Est, ovariectomized 17β-estradiol (30 µg) treated female rats. Values are expressed as DPM/mg (mean ± SEM). * p < 0.05.

does confirm that CART is affected by cocaine. It is possible that alterations in the CART system are more pronounced at later stages of the cocaine abuse cycle. For example, in consideration of the putative anxiogenic effect of CART peptides (Kask et al. 2000; Chaki et al. 2003), there may be long-term neuroadaptations in the CART system during the abstinence phase that is associated to anxiety and depression.

The anxiogenic and locomotor stimulating properties of CART peptides as well as the induction of conditioned place preference (Kimmel et al. 2000) may relate to the gender dependent regulation of CART mRNA expression by cocaine since there are known gender differences in these behaviors. Cocaine-induced locomotor activation and expression of locomotor sensitization is greater in the female than the male rat (van Haaren & Meyer 1991; Hu & Becker 2003). Furthermore, female rats acquire self-administration faster then males (Lynch & Carroll 1999) and are more sensitive to reinstatement after cocaine extinction (Lynch & Carroll 2000).

4.2 COCAINE EFFECTS ON STRIATAL PRODYNORPHIN mRNA EXPRESSION

Cocaine, as a powerful inhibitor of the dopamine transporter, cause elevated extracellular levels of dopamine. In the striatum, dopamine acts on post-synaptic dopamine receptors on medium spiny neurons that express various neuropeptides (Palkovitz et al. 1984; Beckstead & Kersey 1985) such as the opioid peptide dynorphin (DYN). It is well documented that proDYN (PDYN) mRNA expression is upregulated
TABLE 2. Acute effects of cocaine on PDYN mRNA expression in the rat

<table>
<thead>
<tr>
<th>Study</th>
<th>Day</th>
<th>Dose (mg/kg i.p.)</th>
<th>Method</th>
<th>Time of death</th>
<th>CP</th>
<th>DM/L</th>
<th>VM/VL</th>
<th>ACC</th>
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<td>Hurd (-92)</td>
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<td>30 × 2</td>
<td>ISHH</td>
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<tr>
<td>Daunais (-94)</td>
<td>1</td>
<td>10 × 2</td>
<td>ISHH</td>
<td>1 h</td>
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<td>Spangler (96)</td>
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<td>15 × 3*</td>
<td>RPA</td>
<td>30 min</td>
<td>↑</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Spangler (-97)</td>
<td>1</td>
<td>15 × 3*</td>
<td>RPA</td>
<td>30 min</td>
<td>↑</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>24 h</td>
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<td>↔</td>
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</tr>
<tr>
<td></td>
<td>2</td>
<td>30 min</td>
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<td></td>
<td>↑</td>
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<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Turchan (98)</td>
<td>1</td>
<td>20 × 3*</td>
<td>ISHH</td>
<td>3 h</td>
<td>↑</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Svensson (-98)</td>
<td>1</td>
<td>30 × 2</td>
<td>ISHH</td>
<td>2 h</td>
<td>↑</td>
<td>↔</td>
<td>↑</td>
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<tr>
<td>Adams (2000)</td>
<td>1</td>
<td>30</td>
<td>ISHH</td>
<td>30 min</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 h</td>
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<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>

In bold are the results from paper IV, ↔; no difference, ↑; increased PDYN mRNA levels as compared to control, blank; not studied ISHH; in situ hybridization histochemistry, RPA; ribonuclease protection assay, CP; caudate putamen, DM; dorsomedial, DL; dorsolateral, VM; ventromedial, VL; ventrolateral. *, Binge administration, 1 h interval injections.

TABLE 3. Chronic effects of cocaine on PDYN mRNA expression in the rat

<table>
<thead>
<tr>
<th>Study</th>
<th>Day</th>
<th>Dose (mg/kg i.p.)</th>
<th>Method</th>
<th>Time of death</th>
<th>CP</th>
<th>DM/L</th>
<th>VM/VL</th>
<th>ACC</th>
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<tbody>
<tr>
<td>Hurd (92)</td>
<td>7</td>
<td>200 *</td>
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<td>0 min</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Spangler (93)</td>
<td>14</td>
<td>3.3 × 3*</td>
<td>RPA</td>
<td>30 min</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
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<tr>
<td></td>
<td></td>
<td>10 × 3*</td>
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<tr>
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<td></td>
<td>15 × 3*</td>
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<td>↑</td>
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</tr>
<tr>
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<td>8</td>
<td>3 × 15 × 3*</td>
<td>RPA</td>
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<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
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<tr>
<td>Daunais (93)</td>
<td>14-42</td>
<td>17:36-45:87</td>
<td>ISHH</td>
<td>30 min</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
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<tr>
<td></td>
<td></td>
<td>45-87</td>
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<td></td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
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<tr>
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<td>30 × 2</td>
<td>ISHH</td>
<td>30 min</td>
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<tr>
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<td>10</td>
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<td>30</td>
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<td></td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Spangler (96)</td>
<td>14</td>
<td>15 × 3*</td>
<td>RPA</td>
<td>30 min</td>
<td>↑</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
</tr>
<tr>
<td>Mathieu-Kia (98)</td>
<td>14</td>
<td>12 × 3*</td>
<td>ISHH</td>
<td>2 h</td>
<td>↑</td>
<td>↔</td>
<td>↑/↑</td>
<td>↑</td>
</tr>
<tr>
<td>Turchan (98)</td>
<td>5</td>
<td>20 × 3*</td>
<td>ISHH</td>
<td>3 h</td>
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<tr>
<td>Svensson (98)</td>
<td>10</td>
<td>30 × 2</td>
<td>ISHH</td>
<td>2 h</td>
<td>↑</td>
<td>↔</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>

For symbols and abbreviations see Table 2. ↓; decreased PDYN mRNA levels as compared to control, a; self-administration, i.v., b; only the rostral pole was upregulated. Italic indicates withdrawal studies.
Figure 8. The effect of cocaine administration (acute and subchronic) and withdrawal from cocaine on prodynorphin mRNA expression in five subregions of striatum. Acute cocaine: rats were administered one single injection of cocaine (30mg/kg, i.p.) and sacrificed 2 h later. Subchronic cocaine: rats were administered cocaine (30mg/kg, i.p.) once daily for 10 days and sacrificed 2 h after the last injection. Withdrawal cocaine: animals were treated as the subchronic group but were sacrificed after a 10 day withdrawal period. Saline: rats were treated as the acute, intermittent and withdrawal groups but were injected with saline instead of cocaine. The different saline treatments did not produce any significant changes within the regions examined for any of the mRNAs studied, consequently the saline rats were combined to one control group. Values are expressed as DPM/mg (mean ± S.E.M.).* p < 0.05, ** p < 0.01, *** p < 0.001 vs. saline; ° p < 0.05, °° p < 0.01; °°° p < 0.001 vs. withdrawal cocaine.

Prodynorphin

in the striatum after cocaine use in humans (Hurd & Herkenham 1993), as well as after cocaine administration in rodents (see Table 2-3). However, the temporal alteration of the PDYN mRNA expression in the effects of cocaine is not fully investigated. Papers III and IV focus on the DYNergic neuroadaptations at different phases of the cocaine abuse cycle in the monkey and rat striatum.

In paper III, we examined temporal alterations in striatal PDYN gene expression in monkeys after initial and chronic phases of low- and high-dose cocaine self-administration as compared to cocaine-naïve controls. Adult Rhesus monkeys were trained to self-administer food (banana flavored pellets) or cocaine (0.03 or 0.3 mg/kg/inj) on a fixed interval 3-min schedule for 5 or 100 sessions. In paper IV, we investigated the PDYN mRNA expression in rats during abstinence from 10 daily cocaine injections (subchronic administration) as compared to acute (a single injection) and subchronic cocaine administration (30 mg/kg).
Figure 9. Representative autoradiograms of PDYN mRNA expression from a cocaine naïve monkey (control; left) and from a monkey after 100 days of cocaine (0.3mg/kg/inj) self-administration (chronic, high dose; right). Note the enhanced number of visible patches/striosomes and the elevated PDYN transcript levels in the cocaine-exposed animal as compared to control.

Figure 10. Schematic drawing of PDYN mRNA expression changes in the primate striatum after the initial (5 days) and chronic (100 days) phases of low- and high-dose cocaine self-administration (0.03 and 0.3 mg/kg/inj, respectively). Squares represent the integrated densities (dpm/mg X mm²) of the patch/striosome compartment in the dorsal striatum and high-PDYN expressing cell populations of the nucleus accumbens. Surrounding areas represent mean densities (dpm/mg) of low-PDYN expressing cell populations of the dorsal striatum (matrix) and nucleus accumbens, except for the rostral and caudoventral putamen that represent the combined compartments. Colors indicate the percent changes in PDYN mRNA expression densities compared with control values, ns = non significant, trend (p < 0.1), s = significantly (p < 0.05) different from control. Note the elevated integrated density levels in the patch/striosome compartment of all cocaine-exposed animals and the unchanged mean density levels of the chronic low-dose animals.
4.2.1 Elevated expression levels during drug on-board

Consistent with previous studies we found cocaine on-board to cause elevated PDYN mRNA levels in the dorsal striatum. In the rat, primarily acute, but also subchronic cocaine administration resulted in upregulated PDYN expression (see Fig. 8). In the monkey, high- but not low-dose of cocaine resulted in increased PDYN transcript levels in the patch/striosome, but not matrix, compartment following 5 days self-administration, whereas after 100 days of cocaine self-administration, the transcript was upregulated in both striatal compartments (see Fig. 9 and summarized results Fig. 10). These results suggest that the first exposure to cocaine triggers the transcription of PDYN mRNA. After a few exposures, subchronic administration in the rat and initial self-administration in the monkey, the mRNA production is still elevated but to a lesser extent than acutely. The DYN peptide levels are elevated in the striatum, substantia nigra and nucleus accumbens after chronic, but not acute cocaine administration (Sivam 1989; Smiley et al. 1990). Thus, after chronic long-term cocaine exposure, a transition has occurred with a constantly higher DYN tone, at both transcript and presumable peptide levels.

Elevated PDYN mRNA levels have also been documented in human cocaine users (Hurd & Herkenham 1993), but whether the finding is due to the cocaine exposure alone is difficult to interpret since these subjects have varied drug use histories, medical histories and possible preexisting differences in gene transcription. However, in paper III, we studied a primate model of cocaine dependence, which is an optimal model in relation to the human cocaine abuse condition considering the close anatomical homology between the species. Our dose-dependent results in the primate suggest that a history of long-term, high-dose cocaine exposure most likely contributed to the PDYN mRNA alterations found in the human cocaine users. Furthermore, dose-dependent effects on the PDYN mRNA following cocaine use have previously been demonstrated in rats in which similar neuroadaptations were observed only after high-dose exposure or high drug intake (Daunais et al. 1993; Steiner & Gerfen 1993; Daunais & McGinty 1994; but see Spangler et al. 1993).

4.2.2 Temporal responsivity in the primate striatal compartments

The temporal responsivity of the PDYN neuroadaptations to initial and chronic self-administration was confined to the patch/striosome and matrix compartments of the dorsal putamen and caudate nucleus. The different DYNergic anatomical connectivity of the striatal compartments is illustrated in Figure 11 (for review see Steiner & Gerfen 1998). Striatal DYN is suggested to provide negative feedback on dopamine transmission via stimulation of kappa receptors either postsynaptically in the substantia nigra (patch/striosome compartment), or locally in the striatum (both compartments). Thus, the initial elevation of PDYN mRNA expression in the patch/striosome compartment probably reflects neuroadaptation to the cocaine-induced potentiation of dopamine resulting in elevated DYN peptide (Sivam 1989; Smiley et al. 1990; Turchan et al. 1998) to dampen the excessive dopamine overflow in the striatum.
Cocaine effects on striatal dynorphin and CART neuropeptides

Figure 11. Schematic drawing of the DYNergic output projections from the patch/striosome and matrix compartments. 1. Locally released DYN in both striatal compartments via projection collaterals or dendrites, lead to kappa receptor stimulation on dopamine terminals resulting in reduced extracellular dopamine levels. 2. Similarly, DYN release in the substantia nigra compacta (SNc) from patch/striosome projections results in reduced firing of the dopamine neuron and subsequent reduced dopamine release in the striatum. 3. DYN release in the major output region of the matrix, the substantia nigra reticulata (SNr) activates kappa receptors on glutamate terminales. Reduced glutamate release in the SNr inhibits the GABAergic projection to the thalamus (VA, ventral anterior) resulting in disinhibition of thalamic output and subsequently increased locomotor activity via activation of motor cortecies.

In contrast, following chronic self-administration, both the patch/striosome and matrix compartments showed upregulated PDYN transcript levels. Again, these neuroadaptations will result in inhibition of dopamine release via increased DYN tone in both compartments. In addition, kappa stimulation in the major output region of the matrix, substantia nigra reticulata and internal globus pallidum, inhibits the GABAergic projection to the thalamus resulting in disinhibition of thalamic output and subsequently increased locomotor activity (Thompson & Walker 1992; Maneuf et al. 1995). Consequently, the elevated PDYN mRNA expression in the matrix during chronic high-dose cocaine self-administration may be involved in motoric agitations often seen following long-term psychostimulant exposure. In fact, recent studies have shown increased striatal DYN mRNA expression in association with enhanced locomotor activity produced by natural reward or psychostimulants (Werme et al. 2000; Gonzalez-Nicolini & McGinty 2002), as well as repeated L-DOPA administration (Cenci et al. 1998; Andersson et al. 1999). Altogether, the neuroadaptations in the DYN system found in the initial phase may reflect a compensatory action to the altered high dopamine levels, whereas the finding in the chronic phase reflects the neuroadaptation in a brain sensitized to cocaine.
4.2.3 Neuroadaptations in the dorsal versus ventral striatum

Although the nucleus accumbens is considered the major reward brain region and kappa-mediated pharmacological manipulations of cocaine-induced behaviors is suggested to act in this region, the cocaine-induced PDYN neuroadaptations found in paper III were confined to the dorsal, not ventral, striatum. In paper IV, the medial accumbens region was not examined, however most studies to date have found cocaine-evoked activation of the PDYN mRNA expression in the dorsal part of the caudate-putamen after cocaine exposure (see Table 2-3; Hurd et al. 1992; Daunais et al. 1993; Hurd & Herkenham 1993; Spangler et al. 1993; Steiner & Gerfen 1993; Daunais & McGinty 1994; Daunais et al. 1995; Spangler et al. 1996a; Mathieu-Kia & Besson 1998; Svensson & Hurd 1998; Turchan et al. 1998). Upregulated PDYN transcript levels in the nucleus accumbens have mainly been observed after very high doses of cocaine administration (Hurd et al. 1992; Turchan et al. 1998), but Mathieu-Kia et al (1998) observed elevated levels in the rostral pole of the nucleus accumbens following only moderate cocaine dosage.

The heterogeneity of the nucleus accumbens, both in anatomical connectivity and organization of striatal markers (Groenewegen et al. 1999; Furuta et al. 2002) indicate that different neuronal populations within the structure may express different sensitivity to the PDYN neuroadaptations after cocaine. Therefore, we investigated high-, and low-expressing, PDYN cell populations in the accumbens following short-term cocaine self-administration in the rat (Fagergren & Hurd 2001). We found different responsivity between the cell populations: high-, but not low-expressing cell populations were increased following cocaine self-administration (see Fig. 12). This effect was not mirrored in the monkey study; neither high- nor low-expressing PDYN cell populations in the primate nucleus accumbens were regulated by cocaine self-administration at any

![Figure 12](image-url)

**Figure 12.** The effect of cocaine self-administration on prodynorphin mRNA expression in low- (A) and high-expressing (B) cell populations in the nucleus accumbens. The mRNA expression levels were measured within the rostral and caudal nucleus accumbens (+1.8 and +0.7 mm relative to bregma, respectively) from three subregions; core (Co), medial shell (mSh) and lateral shell (lSh). Rats were trained to self-administer cocaine or saline for 7 days (n=6/group). The rats acquired stable cocaine, not saline, self-administration within the 7 day training period and were sacrificed 1 h after the final session. Values are expressed as DPM/mg (mean ± S.E.M.).* p < 0.05, ** p < 0.01 vs. saline.
phase studied. Taken together, the present findings suggest that the dorsal, but not the ventral, striatum is most involved in the PDYN neuroadaptations as a consequence of cocaine administration. These alterations may be relevant for motor functions that are impaired by cocaine use (i.e., tremors, tics, involuntary movements and shakes). The dorsal striatum has recently received attention for its role in the long-term dopaminergic neuroadaptations associated with habitual and drug-seeking behavior (Ito et al. 2002). Thus the potential involvement of the dorsal striatum to dependence should not be ruled out.

4.2.4 Long-term effects of cocaine in the striatonigral pathway

In paper IV, we not only studied the PDYN mRNA expression, but we also examined the mRNA expression of the dopamine D1 receptor that is colocalized with PDYN in the direct striatonigral pathway and the mRNA expression of the D2 receptor and enkephalin which are colocalized in the indirect striatopallidal pathway (see Introduction). As already described, the PDYN mRNA levels were increased after acute and subchronic cocaine administration, however, after a 10 day drug-free period following the subchronic treatment, the levels were conversely down-regulated (see Fig. 8). Similarly, we found the D1 receptor transcript to be elevated acutely and downregulated after abstinence (see Fig. 13). In contrast, we found no change in the D2 receptor mRNA expression during any condition studied. The enkephalin transcript was upregulated following the acute cocaine administration, but the levels were normalized in the subchronic and abstinence phase.

![D1 Dopamine receptor](image.png)

**Figure 13.** The effect of cocaine administration (acute and intermittent) and withdrawal from cocaine on dopamine D1 receptor mRNA expression, in five subregions of the striatum. Details are given in figure 8. Values are expressed as DPM/mg (mean ± S.E.M.). * p < 0.05, ** p < 0.01, *** p < 0.001 vs. saline; ° p < 0.05, °° p < 0.01; °°° p < 0.001 vs. withdrawal cocaine.
Figure 14. The effects of cocaine on the proposed basal ganglia circuitry in different phases of the cocaine abuse cycle. Activation of the striatonigral pathway was found after acute (paper IV, elevated PDYN and D1 receptor mRNA after a single injection in the rat), subchronic (paper III, elevated PDYN mRNA in the patch/striosome compartments after 5 days of self-administration in the monkey; paper IV, elevated PDYN mRNA after 10 days of repeated i.p. injections), and chronic (paper III, elevated PDYN mRNA in both striatal compartments after 100 days of self-administration) cocaine administration. The activation of the direct striatonigral pathway reduces the nigral inhibition of the thalamus resulting in enhanced output signaling that is associated with hypermotor activity. On the contrary, inhibition of the striatonigral pathway was found during abstinence from cocaine (paper IV, reduced PDYN and D1 receptor mRNA after after 10 days of withdrawal from subchronic cocaine administration in the rat). Reduced activity in the striatonigral pathway would result in diminished output signaling via strong inhibition of the thalamus leading to hypomotor activity. The enkephalin (ENK) and D2 receptor mRNAs in the striatopallidal pathway were not as sensitive to the effect of cocaine. An activation was only indicated after acute cocaine (up-regulated enkephalin mRNA in paper IV). These striatopallidal markers were not studied in paper III, but the literature provides evidence for inhibition of this pathway after chronic longterm cocaine exposure (*). CP, dorsal striatum; EP, entopeduncular nucleus (internal globus pallidum in the primate); GP, globus pallidum; SNc, substantia nigra compacta; SNr, substantia nigra reticulata; STN, subthalamic nucleus; VA-VL, ventral anterior, ventral lateral.

Increased densities of D1 receptors, but no change in D2 receptors, have previously been reported after chronic cocaine treatment in the rat (Alburges et al. 1993; Unterwald et al. 1994a). Similarly, the upregulated PDYN mRNA levels following chronic high dose self-administration in the monkey (paper III) was accompanied with increased striatal D1 receptor densities, but decreased D2 receptor densities (Nader et al. 2002). In addition, enkephalin mRNA levels has been reported to be reduced in the Rhesus monkey after two years of cocaine self-administration (Daunais et al. 1997). This pattern of opposing activities of the direct and indirect striatal pathways has also
been demonstrated in human psychostimulant abusers. Hurd and Herkenham (1993) found increased PDYN and decreased enkephalin mRNA levels, and Worsley et al (2000) reported elevated D1 receptor protein and a trend for reduced D2 levels. However no alterations in dopamine receptor densities has also been reported (Meador-Woodruff et al. 1993). Taken together, the present results parallel the published literature of an activation of the striatonigral pathway but not of the striatopallidal pathway to the long-term effects of cocaine.

The striatonigral adaptation to different phases in the cocaine abuse cycle is mirrored in the behavioral effects of cocaine. Acute cocaine administration increases motor activity and repeated cocaine administration produces sensitization of this behavior (Post et al. 1981; Robinson & Becker 1982; Kalivas et al. 1988a). Withdrawal from cocaine in contrast, is associated with depression, which can be expressed as psychomotor retardation. In fact, hypolocomotion has been demonstrated in the rat during withdrawal from cocaine (Fung & Richard 1994; Neisewander et al. 1996) and amphetamine (Pulvirenti & Koob 1993). These behavioral responses are in agreement with our suggestion that the proPDYN- and D1 receptor-expressing striatonigral neurons are important for the actions of cocaine. Based on the proposed basal ganglia circuitry (see schematic illustration, Fig. 14), activation of the striatonigral pathway reduces the nigral inhibition of the thalamus resulting in enhanced output signaling that is associated with hypermotor activity. On the contrary, reduced activation of the striatonigral pathway would result in diminished output signaling via strong inhibition of the thalamus leading to hypomotor activity. Thus, this pattern would be consistent with an up-regulation of PDYN mRNA expression and D1 receptor mRNA or binding sites during acute, subchronic, and chronic cocaine treatment, but a down-regulation during withdrawal.

4.3 COCAINE AND MOOD DISORDER; COEXISTENCE

The apparent coexistence of depression and cocaine dependence suggests shared neurobiological mechanisms underlying these disorders (see Introduction). In this section, behavioral disturbances in relation to the coexistence of cocaine and depression will be investigated. A possible shared neurobiology involving the prodynorphin mRNA expression will also be evaluated.

4.3.1 Experimental anxiety during abstinence (paper V)

The long-lasting neurochemical alterations produced by cocaine may be expressed as changes in emotional behaviors. Each stage in the cycle of cocaine abuse is characterized by specific psychological states. The acute drug on-board state is initiated by euphoria, but at a high dose, or following long-term use, the euphoria is mixed with anxiety and craving (Spotts & Shontz 1984). Abstinence from chronic cocaine abuse, on the other hand, is associated with negative mood states such as anxiety and depression (Gawin & Kleber 1989). In attempt to link behavioral function to the molecular results of the neuroadaptation in the basal ganglia circuitry, the level of anxiety and the basal locomotor activity were investigated in rats during different stages of cocaine withdrawal.
TABLE 4
Effects of withdrawal from chronic binge cocaine on the elevated plus-maze

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<thead>
<tr>
<th></th>
<th>% Time spent open arms</th>
<th>Total number of entries</th>
<th>% Open arm entries</th>
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</thead>
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<tr>
<td></td>
<td>Control</td>
<td>Cocaine</td>
<td>Control</td>
</tr>
<tr>
<td>30 min</td>
<td>32.0 ± 6.3</td>
<td>73.3 ± 5.0***</td>
<td>15.6 ± 1.1</td>
</tr>
<tr>
<td>1 day</td>
<td>42.7 ± 8.0</td>
<td>48.4 ± 7.4</td>
<td>17.0 ± 0.9</td>
</tr>
<tr>
<td>3 days</td>
<td>50.7 ± 6.5</td>
<td>51.5 ± 6.0</td>
<td>15.9 ± 1.1</td>
</tr>
<tr>
<td>10 days</td>
<td>36.2 ± 5.9</td>
<td>52.7 ± 6.2</td>
<td>16.0 ± 1.5</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM. Each group contained 9-10 rats. * indicate difference from control.
* = p < 0.05, ** = p < 0.01, *** = p < 0.001

TABLE 5
Reports on the effect of cocaine on the elevated plus-maze

<table>
<thead>
<tr>
<th>Study</th>
<th>Strain</th>
<th>Treatment</th>
<th>Time of test (post inj)</th>
<th>% Open time control</th>
<th>% Open time cocaine</th>
</tr>
</thead>
<tbody>
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<td>Pellow (85)</td>
<td>HL &gt;250g</td>
<td>1 1 amp 30min</td>
<td>ca 38 ± 7</td>
<td>ca 22 ± 7*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 amp</td>
<td></td>
<td>ca 28 ± 3*</td>
<td></td>
</tr>
<tr>
<td>Yang (91)</td>
<td>CD-1 Mice</td>
<td>1 20 20min</td>
<td>ca 20 ± 4</td>
<td>ca 3 ± 1*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>anxiogenic*</td>
<td></td>
</tr>
<tr>
<td>Rogerio (92)*</td>
<td>Wistar &gt;300g</td>
<td>1 10 15min</td>
<td>3.1 ± 2</td>
<td>2.3 ± 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>42.7 ± 5</td>
<td>14.5 ± 5*</td>
</tr>
<tr>
<td>Sarayai (95)</td>
<td>Wistar &gt;180g</td>
<td>1 20 30min</td>
<td>-</td>
<td>- ns</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>42.7 ± 5</td>
<td>14.5 ± 5*</td>
</tr>
<tr>
<td>DeVries (98)</td>
<td>SD &lt;250g</td>
<td>6 10 30 min</td>
<td>ca 17 ± 3</td>
<td>ca 8 ± 2*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ca 6 ± 2*</td>
<td>ca 2 ± 1*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24h</td>
<td>ca 8 ± 3*</td>
</tr>
<tr>
<td>Basso (99)</td>
<td>Wistar &gt;220g</td>
<td>14 20 48h</td>
<td>52 ± 5</td>
<td>48 ± 4 ns</td>
<td></td>
</tr>
<tr>
<td>Lilly (2000)</td>
<td>SD &gt;130g</td>
<td>14 15 24h</td>
<td>ca 20 ± 6</td>
<td>ca 28 ± 8 ns</td>
<td></td>
</tr>
<tr>
<td>Paine (2002)</td>
<td>LE &gt;250g</td>
<td>1 20 40min</td>
<td>ca 37 ± 5</td>
<td>ca 13 ± 7*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>48h</td>
<td>ca 13 ± 7*</td>
</tr>
<tr>
<td>Paper exp. 1</td>
<td>SD &gt;250g</td>
<td>1 3x10 30min</td>
<td>32 ± 6</td>
<td>73 ± 5*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>24h, 72h, 10days</td>
<td>48-52 ± 6-7 ns</td>
</tr>
<tr>
<td>Paper exp. 2</td>
<td>SD &gt;250g</td>
<td>1 30 30min</td>
<td>58 ± 6</td>
<td>78 ± 3*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10 3x10</td>
<td>77 ± 5*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3x10</td>
<td>69 ± 4 ns</td>
</tr>
</tbody>
</table>

In bold; tests during withdrawal. * Rats were exposed twice to the plus-maze test. A selection procedure, in which rats were divided into groups of high or low activity on the plus-maze, was performed prior to the test on the effect of cocaine. SD, Sprague Dawley; HL, Hooded Lister; LE, Long Evans; amp, amphetamine. *, p < 0.05; ns, not significant
We did not find experimental anxiety in rats after 1, 3 or 10 days of abstinence from 10 days “binge” cocaine administration (3 x 10 mg/kg). These rats exhibited similar behaviors on the elevated plus-maze (time spent on open arms and number of open or total entries) as their respective saline controls (Table 4). There are two possible interpretations of these results. First, this cocaine administration paradigm was not sufficient to produce abstinence in the rat. In agreement, the lack of anxiogenic effect was accompanied by no change in basal locomotor activity, although hypolocomotion usually is associated with a negative mood state. A second interpretation would be that the rats were abstinent, but we were unable to demonstrate the anxiogenic behavior in our experimental setup. Anxiety and depression in humans consists of complex emotional states that may simply not be expressed as the animal behaviors determined in the present study. In fact, experimental anxiety has been demonstrated following cessation of repeated cocaine administration using the conflict paradigm (Fontana & Commissaris 1989), the light-dark test box (Costall et al. 1989), the defensive burying paradigm (Harris & Aston-Jones 1993; Basso et al. 1999), whereas results from the elevated plus-maze are conflicting.

In Table 5, all reports to date on plus-maze behavior following cocaine administration are listed. Sarnyai (1995), DeVries (1998) and Paine (2002) found anxiogenic effects after abstinence from repeated cocaine administration, whereas Basso (1999) and Lilly (2000) reported no change. Furthermore, we were unable to demonstrate the well-documented anxiogenic effect during cocaine on-board (Pellow et al. 1985; Rogerio & Takahashi 1992; Yang et al. 1992; DeVries & Pert 1998; Paine et al. 2002), but see (Sarnyai et al. 1995; Lilly & Tietz 2000). We found acute and chronic cocaine on-board to result in anxiolytic-like behavior on the elevated plus-maze (see Table 6). To our knowledge this is the first report published on anxiolytic effects of cocaine, although Lilly et al (2000) observed a nonsignificant anxiolytic tendency after chronic cocaine. The discrepancies of the plus-maze behaviors following cocaine administration may be due to differences in the rodent strain examined, drug treatment protocols, and testing conditions of the plus-maze, or a combination of these factors. Wahlsten et al (2003) recently demonstrated that the outcome of the elevated plus-maze behavior depends upon genotype and the laboratory in which testing was performed. In particular, the averseness of the test condition will influence the outcome (Hogg 1996).

<p>| TABLE 6 |
| Acute effects of cocaine on the elevated plus-maze. |</p>
<table>
<thead>
<tr>
<th>N</th>
<th>% Time spent open arms</th>
<th>Total number of entries</th>
<th>% Open arm entries</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>57.7 ± 5.9</td>
<td>16.9 ± 1.4</td>
</tr>
<tr>
<td>Acute single cocaine</td>
<td>10</td>
<td>77.8 ± 2.6*</td>
<td>19.9 ± 1.9</td>
</tr>
<tr>
<td>Acute binge cocaine</td>
<td>8</td>
<td>76.8 ± 5.4*</td>
<td>20.1 ± 2.9</td>
</tr>
<tr>
<td>Chronic binge cocaine</td>
<td>6</td>
<td>69.0 ± 4.2</td>
<td>16.8 ± 0.9</td>
</tr>
</tbody>
</table>

Testing was performed 30 min after the last injection. Values are expressed as mean ± SEM. * indicate difference from control. *= p < 0.05.
In conclusion, we failed to demonstrate anxiogenic effects of cocaine both during drug on-board and after abstinence, suggesting that experimental anxiety as measured on the plus-maze is not a major component of cocaine administration using this experimental design.

**4.3.2 Cocaine effects in an animal model of depression (paper VI)**

In the previous section, we investigated whether repeated cocaine administration would induce a negative mood state. Here we used an opposite approach: does an underlying negative mood state have an effect on the responsivity to cocaine. As such, we investigated the influence of a depression-related genotype on the effects of cocaine by using an animal model of depression, the FSL rat. First, attempts were made to evaluate the rewarding properties, whether a depression genotype facilitate (indicative of self-medication) or reduce (expression of anhedonia) the development of cocaine dependence by studying cocaine self-administration behavior. Second, we examined novelty behavior and the locomotor stimulating effect of cocaine simultaneous with \textit{in vivo} mesolimbic dopamine overflow.

**4.3.2.1 Cocaine self-administration behavior**

We found no difference in cocaine intake between the FSL and control FRL rats during the first ten days of cocaine self-administration at a FR-1 schedule of reinforcement (1.5 mg/kg/inj; see Fig. 15a). However, in this study a high dose of cocaine was used, whereas different propensities to acquire self-administration behavior between rats have been reported at lower doses of cocaine (Matthews et al. 1999; Mantsch et al. 2001). In agreement, a within dose-response function (0.02, 0.09, 0.38, 1.5 mg/kg/inj) revealed different sensitivity between the Flinders rats at a lower dose (see Fig. 15b). We found a subtle reduction in cocaine reinforcement in the FSL rat; the cocaine intake was reduced at one dose studied (0.09 mg/kg/inj). In agreement, Overstreet et al (Overstreet et al. 1992) reported no difference in alcohol preference and no change or reductions of reward have been reported from anhedonia models in the FSL rats (see Introduction; Pucilowski et al. 1993; Ayensu et al. 1995; Matthews et al. 1996).

Limited studies to date have investigated cocaine responsivity in animal models of depression. Reports stemming from maternally separated animal studies have been conflicting, showing both reduced and enhanced acquisition of cocaine during self-administration (Matthews et al. 1999; Kosten et al. 2000). Additionally confounding, the olfactory bulbectomized rats were found to have increased self-administration behaviors as compared to controls (Holmes et al. 2002).

**4.3.2.2 Locomotor activity and behavioral responses**

In contrast to the subtle cocaine self-administration differences found in the Flinder rats, there were significant differences in the behavioral responsivity to novelty and cocaine administration (30 mg/kg for 10 days). The FSL rats showed reduced horizontal activity during the first 30 min of novelty as compared to the FRL rats (Fig. 16a). In addition, the FSL rats exhibited less grooming behavior (1.2 ± 0.6 vs. 3.3 ± 0.2,
Cocaine effects on striatal dynorphin and CART neuropeptides

Figure 15. A) Acquisition of i.v. cocaine self-administration at the dose of 1.5 mg/kg/inj in FSL (n = 11) and FRL (n = 9) rats. Data are presented as the total reinforcers of a 3h session of cocaine self-administration across the first ten days of acquisition. B) Within session cocaine dose-response function for the FSL (n = 8) and FRL (n = 9) rats. Rats had access to each dose for 45 min, starting with the high training dose at 1.5 mg/kg/inj, followed by 0.375, 0.094, 0.023 mg/kg/inj. Data are presented as number of reinforcers from the last 30 min of each dose-exposure. Data are expressed as mean ± SEM. * indicate day differences from baseline (day 1), † indicate rat line differences. * = p < 0.05, ††, ** = p < 0.01 *** p < 0.001.

*p < 0.001) and produced more fecal boli (7.4 ± 1.6 vs. 3.7 ± 0.6, p < 0.05) versus control. These results are in line with hypolocomotion in depressed individuals and increased stress-reactivity, which are documented behavioral traits of the FSL rats (see Overstreet 1993). In addition to the low novelty response, the FSL rats exhibited less locomotor activation after repeated cocaine administration than their controls. Both Flinders rat lines showed elevated horizontal activity after the cocaine injection as compared to their respective saline animals, but the duration and magnitude was
Figure 16. Horizontal activity (A) and forward locomotion (B) during novelty, 0-60 min, and following an injection of cocaine (30 mg/kg/inj) or saline, 60-160 min. Data are expressed as mean ± SEM, † indicate rat line differences, # indicate time differences within each rat line, + indicate treatment differences within each rat line, and * indicate time differences from baseline. †, +, # = p < 0.05, ††, ++, *** = p < 0.01, +++ = p < 0.001.

blunted in the FSL rats (Fig 16a). Furthermore, the FSL rats exhibited less forward locomotion as compared to the FRL rats after the injection (Fig 16b). In contrast, the stereotypy was increased in the FSL rats (6.1 ± 0.3 vs. 5.3 ± 0.2, p < 0.05, Whitney U test). Both lines expressed intense sniffing and head bobbing; however, the FSL rats had greater stereotypy scores due to their very restricted locomotion. Different interpretations might be drawn for these cocaine-induced behaviors: does the FSL rat exhibit a blunted or sensitized locomotor response? Normally, cocaine produces locomotor activation that becomes sensitized with increasing doses or after repeated administrations of the drug until stereotypies start to develop. Our current dosing procedure most likely leads to sensitization. However, the enhanced stereotypy in the
FSL rats could be due to their lower basal locomotion or to a greater responsivity to cocaine, i.e. a heightened shift towards stereotypy at a lower threshold. We therefore examined the locomotor stimulating effect at a lower dose of cocaine (15mg/kg; which does not produce stereotyped behavior), after acute and repeated (7 days) administrations. We found a trend towards reduced horizontal activity in the FSL rats after the first injection (p = 0.078, Fig. 17a), but there was no difference between the lines after the seventh injection (Fig. 17b). Furthermore, both Flinders strains exhibited similar forward locomotion after acute and repeated cocaine administration.

The blunted response to acute low dose cocaine is consistent with the reduced locomotion following acute d-amphetamine administration, as demonstrated by Jimenez Vasquez et al (Jimenez Vasquez et al. 2000). However, the FSL rats may sensitize more to repeated cocaine as compared to the control rats considering their similar locomotor response following repeated low dose cocaine. It is clear that the two
strains show qualitatively different responses to cocaine administration. Taken together, the data suggests that the FSL rats are more susceptible to the sensitizing effects of intermittent cocaine administration.

4.3.2.3 Mesolimbic dopamine overflow

Although we found marked alterations in dopamine-mediated behaviors between the Flinder lines, there were no significant differences in dopamine levels in the nucleus accumbens shell during baseline, novelty, or after cocaine administration. This contrasts other studies that have reported reduced in vivo basal dopamine and metabolite levels in the nucleus accumbens (Yadid et al. 2001; Zangen et al. 2001) and caudate-putamen (Auta et al. 2000) of the FSL rats. The discrepancies may be due to the striatal subregions examined and to different control animals used in these studies. The original FRL control rats that were bred parallel to the FSL rats were used in the current study, whereas Yadid et al. (2001) and Zangen et al. (2001) used non-parallel bred Sprague Dawley. Furthermore, cocaine stimulation was not accompanied with significantly differentiated dopamine overflow between the Flinder lines in the nucleus accumbens shell, but we cannot discount possible dopamine alterations in e.g., the more motor-related nucleus accumbens core or the caudate-putamen (Heimer et al. 1991; Zahm & Brog 1992a). The differentiated locomotor behavior can also depend on postsynaptic responses to the dopamine overflow such as alterations in e.g., receptor activity and 2nd messenger systems. Future studies will be carried out to determine the possible differences in postsynaptic activity of the dopamine system.

4.3.2.4 Individual vulnerability to psychostimulants

Individual vulnerability to psychostimulants can be predicted by the response to novelty (Piazza et al. 1989). We found that the FSL rats exhibit lower horizontal locomotor activity than controls in a novel environment that makes them low responders to novelty. It is well-documented that high responders to novelty more readily acquire low dose amphetamine or cocaine self-administration as compared to low responders (Piazza et al. 1990; Deroche et al. 1995; Grimm & See 1997; Pierre & Vezina 1997; Marinelli & White 2000; Piazza et al. 2000; Mantsch et al. 2001), and recently high responders were found to display an upward shift in the cocaine dose-response curve (Piazza et al. 2000). In addition, high responders show enhanced locomotor response to acute psychostimulant administration (Hooks et al. 1991; Exner & Clark 1993) and increased cocaine-induced DA overflow in the nucleus accumbens (Hooks et al. 1991; Hooks et al. 1992). This suggests that high novelty responders are more vulnerable to the reinforcing effect of psychostimulants. Consistently, low responders such as the FSL rats may be less vulnerable to cocaine. Although, both Flinders rats acquired self-administration at similar rates using a high dose of cocaine, the change at a low dose in the dose-response curve may indicate a reduction in cocaine reinforcement at lower doses in the FSL rats. Furthermore, the FSL rats showed reduced locomotor response to acute psychostimulant administration (Jimenez Vasquez et al. 2000; present results). Taken together, a depression genotype appears to be associated initially with decreased vulnerability and locomotor responsivity to psychostimulant drugs.
Mood disorder and striatal prodynorphin mRNA expression

The postulated shared neurobiological mechanisms underlying the coexistence of depression and cocaine dependence may involve the DYN system. This section describes our recent investigation of the striatal PDYN mRNA levels in the Flinders animal model of depression.

We compared the basal expression of PDYN mRNA in the Flinders rats using in situ hybridization histochemistry. The FSL rats showed reduced PDYN levels in the caudal striatum as compared to control FRL rats (Fig 18; dorsal, p = 0.008; ventral, p = 0.03; FSL, n = 4; FRL, n = 5), but no difference in the rostral subregions of the striatum, including nucleus accumbens shell and core, as well as caudate-putamen patch/striosome and matrix compartments. These results are in line with the down-regulated PDYN transcript found during cocaine abstinence (paper IV), although the significant differences were found in different subregions within the striatum. It is still interesting that the changes found points to the same direction, namely a lower DYN tone in association to an apparent depressed mood state. Considering the postulated dopaminergic hypofunction during depression and cocaine abstinence, we suggest that low dopamine levels results in suppression of the striatonigral DYN tone as a compensatory action to increase striatal dopamine levels (see Fig. 8). Furthermore, the confinement of the PDYN reductions to the motor-related dorsal striatum suggests impairments in motor function. Interestingly, impaired DA function has been found in the dorsal striatum of human depressed subjects (Martinot et al. 2001; Meyer et al. 2001). In fact, Martinot et al (2001) demonstrated a direct link between dopamine hypofunction and psychomotor retardation in these subjects. As discussed in section 4.2.4 regarding the basal ganglia circuity, enhanced locomotor activity is associated with upregulated PDYN mRNA expression, whereas reduced locomotor activity may be associated with down-regulated PDYN mRNA expression. In agreement, hypolocomotion is a behavioral trait of the FSL rats (see Overstreet 1993), which was demonstrated in paper VI during novelty.

Figure 18. Representative autoradiograms of PDYN mRNA expression in coronal sections from FRL (to the left) and FSL (to the right) rats. Note the reduced expression in the caudate-putamen of the FSL rats. Ce, central amygdala; CP, caudate-putamen; DG, dentate gyrus; PVN, paraventricular nucleus.
In contrast to the reduced PDYN mRNA levels found in the caudal striatum of the FSL “depressed” rats, an increased DYN tone has been suggested to underlie a negative mood state (see Introduction). Based on the anatomical connectivity of the striatum, different sites of DYNergic action within this structure possibly mediate different functions. The suppressed PDYN mRNA levels, associated with negative affect, demonstrated in this thesis work, were found in striatal subregions relevant for motor function. On the contrary, we found no changes in the PDYN expression in the limbic-related subregions of the striatum relevant for motivation and reward.
5 SUMMARY

- Human CART mRNA expression was to a great extent confined to brain regions related to cocaine dependence, including most target regions of the mesocorticolimbic dopamine pathway and regions in the ventral striatopallidal circuitry.

- CART mRNA expression is regulated by acute cocaine administration. Although we did not confirm the original finding with increased levels in the striatum of the male rat, we found up-regulations in the male central amygdala and the female nucleus accumbens shell.

- We found dose-dependent and temporal upregulation of the dorsal striatal PDYN mRNA in monkeys that had self-administered cocaine. The patch/striosome compartment was initially more sensitive to the induction of the PDYN gene transcription with a progression to the matrix with long-term high-dose cocaine self-administration.

- The striatonigral pathway is more sensitive as compared to the striatopallidal pathway to the long-term effects of cocaine. We found suppression on PDYN and D1 receptor systems during withdrawal from cocaine in the rat, but no change in the striatopallidal enkephalin/D2 receptor system.

- We failed to demonstrate anxiogenic effects during withdrawal from "binge" cocaine administration, suggesting that anxiety as measured on the plus-maze is not a major component of cocaine abstinence using this experimental design.

- The Flinders animal model of depression demonstrated a modest decrease in cocaine reinforcement using the self-administration paradigm. In addition, the FSL rats were low responders to novelty, which is associated with decreased vulnerability to addictive drugs.

- The FSL rats may have a more vulnerable phenotype to the behavioral sensitizing effects of intermittent cocaine administration, but a similar dopaminergic response in the nucleus accumbens shell as compared to control animals.

- Both cocaine abstinence and a depression phenotype are associated with reduced PDYN mRNA levels in the rat dorsal caudate-putamen.
6 CONCLUDING REMARKS

Much remains to be examined regarding the involvement of the CART system in cocaine dependence and mood disorders. However, based on the studies presented in this thesis it is clear that the CART transcript is expressed in brain regions relevant for both disorders in the human brain. The expression of CART mRNA was predominately localized to target regions of the mesocorticolumbic dopamine pathway that is considered the major brain reward circuitry. Most drugs of abuse, including cocaine, activate this circuitry and impairments in the pathway are suggested to underlie anhedonia and lack of motivation that are common traits in depressed individuals. CART peptides have been found to activate the mesocorticolumbic dopamine pathway after intra-VTA injections in the rodent. The exclusive localization of CART mRNA in the nucleus accumbens shell of the human striatum is of particular interest, since this region is highly implicated in drug abuse. In addition, many regions included in the greater limbic lobe showed CART mRNA expression. Both cocaine dependent and depressed subjects demonstrate impairments in emotional processing, in which the greater limbic lobe and connected regions plays a role. In agreement, emotional expression in terms of anxiety has been induced by CART peptides in the rat. Similarly the high expression of CART mRNA in hypothalamic nuclei is relevant for these disorders since energy homeostasis and stress responsivity usually are affected. The functional involvement of CART in the hypothalamus is well substantiated by rodent studies demonstrating that CART peptides are anorexic and influence the regulation of the stress hormone CRF.

Although our studies do not confirm the original report with psychostimulant-induced upregulated striatal CART mRNA expression in the male rat, we observed gender specific up-regulations after acute cocaine injections. Further studies are needed to evaluate the involvement of the CART system in the cocaine abuse cycle. CART peptides exhibit psychostimulant properties, such as induction of locomotor stimulation, conditioned place preference, anxiety, and food intake inhibition. Therefore it is possible that antagonism of the CART system would be a potential pharmacological intervention approach for cocaine dependence and perhaps mood disorders. However, the CART receptor/s, together with specific agonists and antagonists, needs to be identified before pharmacological manipulations of the CART system can be thoroughly evaluated.

The short- and long-term alterations of prodynorphin mRNA levels in the monkey following high dose cocaine self-administration were confined to the dorsal striatum. Dopaminergic alterations in the dorsal striatum have been associated with habitual drug-seeking behavior. It has been argued that the ventral striatum is important for the initiation of a drug dependent state but the dorsal part is involved in the maintenance. However, we found the limbic-related patch/striosome compartment to be most sensitive during the initial self-administration phase, indicative of a counteradaptive process to dampen excessive dopamine levels in the dorsal striatum. After 100 days of high dose cocaine self-administration, the matrix compartment also showed elevated
prodynorphin levels that may reflect a pathological state not only involving the
dopamine stimulation but also the output of the basal ganglia, resulting in maladaptive
motor behaviors. Consequently the dynorphinergic neuroadaptations in the motor-
related dorsal striatum following long-term cocaine exposure may be associated with
motor impairments often observed in cocaine dependent subjects.

Both cocaine abstinence and a depression phenotype were associated with reduced
prodynorphin mRNA levels in the caudate-putamen suggesting a low striatal dynorphin
tone during a negative mood state. Considering the anatomy of the basal ganglia
circuitry, low dynorphin activity may underlie hypolocomotion. Locomotor retardation
is a common symptom in depressed individuals and is observed during abstinence from
addictive drugs. In contrast, in the mesocorticolimbic system increased dynorphin
activity is suggested to mediate dysphoria. Although the observed reduction of the
prodynorphin mRNA levels was found in the dorsal striatum during a negative mood
state, we cannot exclude an alteration of the dynorphin system in the ventral striatum.
First, we did not study the medial accumbens during cocaine abstinence as it was only
studied in the Flinders depressed rats where it was unchanged. Second, we only studied
the mRNA expression of the prodynorphin gene. Therefore it is possible that the actual
dynorphin peptide levels or release are altered in the ventral striatum during a negative
mood state. Third, the kappa receptor that mediates the effect of dynorphin may be
altered in these states.

We found subtle signs of anhedonia in the Flinders animal model of depression. In
agreement, no change or reductions of reward have been reported from anhedonia
models and alcohol preference in the FSL rats. The fact that the FSL rats were low
responders to novelty, which is associated with reduced stimulant self-administration
responsivity as well as stimulant-evoked locomotor activation following acute
administration, also adds to a suggested initial reduced reward to cocaine. However, the
FSL rat showed a very different behavioral response to repeated cocaine administration
indicative of a more vulnerable phenotype to the sensitizing effects. The sensitization
process is suggested to underlie the transition to drug dependence. However, the
impaired motor functions after repeated cocaine administration were not correlated to
mesolimbic extracellular dopamine levels. Taken together, definite conclusions about
the reinforcing efficacy of cocaine in the FSL rat will require further studies. It is
nevertheless clear that the different strains show a differential response to cocaine, a
phenomenon also present in human cocaine abusers where a negative mood state most
often is associated with enhanced subjective effects after cocaine use.
7 ACKNOWLEDGEMENTS

Above all, I thank my mentor and guide in the world of neuroscience, professor Yasmin Hurd, for your incomparable supervision, exceptional scientific knowledge, and your infectious enthusiasm as well as laughter. I am very grateful for these years, working with you has been lots of fun. Thanks for support at all levels, from science to babysitting.

To all former and present members of the Hurd-herd, Barbro “Babben” Berthelson for your wonderful personality, your respect (read fear) of my pickiness at the lab and your lovely smile. Lauran “Bingolotto” Caberlotto for the best Godisgris roommate ever and Marie “Östrogen” Österlund for valuable lab-support and fun discussions. Maria “Braja-Maja” Stridh, my first-class travel mate, for all pronounced words and for being extremely helpful. Hanna “Snövit” Östlund (Östrogen II) for your infectious laughter. “Speedy”-Nitya Jayaram-Lindström, my caring psychologist for better and for worse. Snyggingen “Party/Puppy”-Parisa Zarnegar my favorite “colleague”. Pauline (Östrogen III) Flodby for your control over the American English and this “book” (should be thesis). Xinyu Wang for adding testosterone to the group. Marja Ponten for introducing me to the world of albino rats. Björn Schilström for all interesting reading in the paper-waste, sorry, recycling. HongMei Yan and Annika Berge for your helping hands. Andrej Nikosjokov for the “Russian grave”. Marita Signarsson the best organizer ever.

All people in the psychosis corridor, for making up a foundation of harmonic working atmosphere, for all discussions and laughter at the breakfasts and coffee breaks, and of course, for the coffee bread. The former and present members of the Hubin group; Prof. Göran Sedwall, Håkan Hall, Katarina Varnås, Alexandra Tylec, Stefan Pauli, Erik Jönsson, Ingrid Agartz, Roger Hult, Henrik Ahlgren, Hebert Corbo, Monika Hellberg, Emma Bonnet, Carina Schmidt, Andreas Ekholm, Kjerstin Lind, Pontus Stålin, Ulrika Kahl, Birgit Engman Shahin Arshamian, Emelie Jönsson, and Siv ”the Viking” Eriksson.

Och annat löst folk: Marianne Youssefi, Lisbeth Eriksson, Nils Lindefors, Diana Radu, Olof Zachrisson, Camilla Lundkvist, Johan Sandell, Oliver Langer, Phong Truong and others members of the Christer Halldin radiochemistry group and the Lars Farde PET-group.

The Pharmacology people, Prof. Urban Ungerstedt, Åse Elfing and Michel Goiny my HPLC advisors, Monica Pace-Sjöberg, Johan Ungerstedt, Neda Rajamand, Natalie Wisniewski, and Silvia DiMaio. Thanks for letting me into your group.

Markus Heilig for introducing me to experimental drug abuse research and to my mentor Yasmin, and the “Heilig family” for recognizing anxious rats.
International collaborators, Prof. David Overstreet, Prof. Mike Nader, Prof. Linda Porrino, Hillary Smith, Susan Nader, Jim Daunais. Thank you for all email correspondence and fun times at meetings.

The Joban Franck addictive group, Lotta Arborelius depressed group, Henrik Druid and Eva Keller dead groups for interesting discussions at seminars and journal clubs.

KI friends; Thank you Alko-Sara my "På Stan Guide" and Knarko-Åsa for our dynorphinergic discussions and friendship. Nicko-Nina for sharing theories of life and of the self-administration paradigm. Annika Thorsell and Lisa Wiklund for fun times in and outside the Magnus Huss Clinic. Ingrid Dahlin for all chats.

My normal friends, Lasse for always showing up in time for sunday dinner, Pernilla for all laughter and babysitting, Cecilia and Monica too far away, Ume-folket, where did time go? Members of the Girl-dinners. The Gangs of Saltsjöbaden and all the rest.


Min Fritiof, det bästa jag någonsin gjort! ❤
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Cocaine effects on striatal dynorphin and CART neuropeptides


