Investigation of the neuropharmacological mechanisms of barbiturate reinforcement using the conditioned place preference paradigm

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Abstract

Drugs of abuse are thought to have the common action of increasing dopaminergic transmission in the mesolimbic dopamine (DA) pathway, specifically in the nucleus accumbens (NAc). However, sedative-hypnotics, including the barbiturates, are anomalous in that they appear to stimulate DA release from the NAc at doses that are generally lower than their reinforcing doses. The fact that barbiturates have a long history of abuse in humans and are potent reinforcers in laboratory animals, but are behaviourally reinforcing only at doses that decrease DA release from the NAc, raises questions about the neuropharmacological mechanism of reinforcement in these drugs. Indeed, of the numerous studies that have examined the reinforcing properties of barbiturates, none have examined the pharmacological basis of their reinforcing effects.

The conditioned place preference (CPP) paradigm is a widely used behavioural test that assesses the reinforcing capacity of stimuli by the ability of conditioned stimuli to evoke an approach response. Using this paradigm, systemic administration of pentobarbital (15 mg/kg) induced a significant place preference. Furthermore, pretreatment with $GABA_A$, DA, and opioid receptor antagonists blocked the pentobarbital-induced place preference. Sodium barbital, a longer-acting barbiturate also induced a significant CPP when systemically administered (8 and 24 mg/kg). Moreover, the reinforcing effect of this place preference is centrally mediated, assessed by the significant CPP obtained with intracerebroventricular (ICV) injections of barbital (240 and 480 μg).

A number of different brain sites are involved in the reinforcing effects of drugs of abuse. Microinjections of barbital into the periaqueductal gray (25 μg) or posterior ventral tegmental area (VTA; 15 μg), but not into other areas, such as the amygdala and anterior VTA, produced a place preference. Furthermore, opioid (naloxone methiodide) and $GABA_A$ receptor (SR 95531) antagonists administered into these areas blocked the ICV barbital place preference. Given these findings, barbiturate reinforcement appears to be mediated by the same neural substrates and neurochemical systems as other drugs of abuse, such as opiates and ethanol. The implications of these results and the use of barbital in the place preference paradigm to investigate the neuropharmacological mechanisms of barbiturate reinforcement are discussed.

Résumé

Les drogues à usage abusif agissent sur le système mésolimbique dopaminergique, plus spécifiquement sur le noyau accumben (NAc), en augmentant la libération de dopamine (DA). Les sédatifs-hypnotiques, dont les barbituriques, sont dits anormaux puisqu'ils stimulent la libération de DA du NAc à des doses moindne que celles provoquant habituellement un renforcement. Chez l'humain, les barbituriques présentent une longue histoire d'abus; chez les animaux de laboratoire, ils sont de poissants reforçateurs. Cependant, le fait qu'ils agissent en tant que reforçateurs uniquement à des doses provoquant une diminution de libération de DA du NAc, soulève des questions sur les mécanismes neuropharmacologiques sous-jacents audit renforcment induit par ces chogues. Jusqu' à maintenant, aucune recherce n`a porté sur les bases pharmacologiques de ces effets renforçateurs.

Le paradigme béhavioral de préférence localisée conditionnée (PLC) est utilisé pour èvaluer l'effet renforçateur de stimuli par la capacité d'un stimulus conditionné à produire une résponse d'approche. Avec ce paradigme, l'administration de penthiobarbital (15 mg/kg) induit une CPP significative. Par ailleurs, un prétaitement auz antagonistes des rècepteurs $GABA_A$, DA, et opioïdes inhibe la CPP normalement induite par penthiobarbital. Le sodium barbital, un barbiturique à effet prolongé, provoque aussi une CPP significative si administré de façon systémique (8 et 24 mg/kg). De plus, une CPP significative est induite par injections intracèrébro-ventriculaires (ICV) de barbital (240 et 480 μg) ce qui suggère un mode d'action central.

Les effets renforçateurs des drogues à usage abusif se manifestent à différentes régions du cerveau. Des micro-injections dans les régions péri-aquducale grise (PAG; 25 μg) et postéro région ventrale tegmentaire (RVT; 15 μg) produisent une CPP. Cet effet n'est par contre pas obesrvé dans les régions amygdalaire et VTA antérieure. De plus, l'administration d'antagonistes aux récepteurs opioïdes (nalaxone méthiodide) et $GABA_A$ (SR 95531) dans les régions PAG et postéro RVT inhibe la CPP ICV induite auparavant par barbital. L'implication de ces résultats ainsi que la justification des barbituriques pour l'étude des mécanismes neuropharmacologiques impliqués dans le renforcement y sont discutès.

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Statement on Research Contributions

Some of the work presented in this dissertation has been previously published in Bossert & Franklin (2001) Pentobarbital-induced place preference in rats is blocked by GABA, dopamine, and opioid antagonists, Psychopharmacology, 157(2): 115-122. In terms of my contributions, I performed all of the behavioural testing, assisted with the design of the experiments and data analysis, and wrote the manuscript under the guidance of my supervisor, Keith Franklin.

CHAPTER 1:

INTRODUCTION

Almost half a century ago, Olds and Milner (1954) observed that rats would work for electrical stimulation in certain areas of the brain. This discovery was a significant breakthrough for behavioural neuroscience, and introduced the concept that there is a specialized brain circuitry that mediates the behavioural effects of reward or reinforcement. By definition, a reinforcer maintains or increases the probability that a response which precedes the reinforcer will subsequently recur. Many contemporary researchers refer to Thorndike's "Law of Effect" as the cornerstone of reinforcement theory. Thorndike believed that behaviour was predictable and that learning was strengthened or weakened when it was accompanied, or closely followed by, satisfaction or discomfort, respectively (Thorndike, 1965). The idea was that reinforcement succeeded the presentation of an appetitive stimulus (positive reinforcement) or the removal of an aversive stimulus (negative reinforcement). Drugs of abuse are considered to be positive reinforcers because they promote self-administration and can promote approach (i.e. drug-seeking) behaviour by their learned associations with stimuli in the environment.

While the various drugs of abuse have different molecular targets, they are thought to have the common action of increasing dopaminergic transmission in the mesolimbic dopamine (DA) pathway, specifically in the nucleus accumbens (NAc) (Di Chiara and Imperato, 1988; Robinson and Camp, 1991). This commonality of action supports the widely held view that the mesolimbic DA system has a general role in the reinforcing effects of drugs. Furthermore, it has been hypothesized that a preferential increase in DA release from the NAc is related to the addictive properties of drugs (Di Chiara, 1995).

However, some drugs with addictive properties may not fit this model. In particular, sedative-hypnotics, including the barbiturates, appear to stimulate DA release from the NAc only at very low doses that stimulate behavioural activity (0.75 mg/kg) and decrease DA release at higher doses (5 mg/kg) (Di Chiara and Imperato, 1986). There is evidence that discrepancies exist between the DA-releasing dose of pentobarbital and doses that are reinforcing in laboratory animals. For instance, while a dose that stimulates DA release from the NAc falls within the range of unit doses that animals will self-administer pentobarbital (0.25 - 4.0 mg/kg/infusion) (Winger et al., 1975; Collins et al., 1984; DeNoble et al., 1985), the amount of drug that is actually infused within a self-administration session is much higher than the amount infused per injection. Moreover, the dose reported to stimulate DA release is 10-20 times lower than the dose of pentobarbital that is reinforcing in brain stimulation experiments (5 - 10 mg/kg; Bossert and Franklin, unpublished data; Seeger et al., 1981) and the place preference task (15 mg/kg; see Chapter 2). The fact that barbiturates have a long history of abuse in humans and are potent reinforcers in laboratory animals, but are reported to be behaviourally reinforcing only at doses that decrease DA release from the NAc, raises questions about the neuropharmacological mechanism of reinforcement in these drugs. In fact, there have been very few studies that have examined the neurochemical effects of barbiturates. Of those studies, most have investigated the anesthetic or chronic effects (i.e. tolerance, dependence) of barbiturates on molecular targets or neurotransmitter systems. Moreover, of the numerous studies that have examined the reinforcing properties of barbiturates, none have examined the pharmacological basis of those reinforcing effects.

This dissertation is an investigation of the neuropharmacological mechanisms of barbiturate reinforcement, and examines whether barbiturates act on the same neurochemical systems and neural substrates as other drugs of abuse. The following introductory sections will (1) review the neuroanatomical sites that are involved in drug reinforcement, (2) discuss the neuropharmacological mechanisms of the major drugs of abuse, (3) outline the history of barbiturate use and review the literature on the reinforcing effects of barbiturates drugs in both humans and laboratory animals, and (4) review the known pharmacological characteristics of barbiturates.

1.1. Neuropharmacology of Reinforcement

Extensive mapping studies of intra-cranial self-stimulation (ICSS) sites in the rat brain led to the identification of neuroanatomical substrates involved in reinforcement processes. Olds (1956) reported that a number of brain areas supported self-stimulation, including the septal area, the amygdaloid complex, and the anterior hypothalamus. Subsequent reports indicated that self-stimulation can be obtained in sites from the olfactory bulb to the mylencephalon (nucleus tractus solitarius) (see Phillips and Fibiger, 1989), however, electrode placements along the medial forebrain bundle (MFB) between the rostral hypothalamus and the ventral tegmental area produce the strongest reinforcing effects.

Identification of this endogenous circuitry led researchers to examine the pathways that contribute to these reinforcing effects and the neurochemical systems that may be

involved. Dahlstrom and Fuxe (1964) identified several clusters of catecholamine-containing (e.g. dopamine, norepinephrine) cell bodies and traced the primary axonal projections of these cell groups to various brain regions, while later studies provided a more precise anatomical localization of these cells and their projections (Lindvall and Bjorklund, 1974). DA neurons projecting to the forebrain originate from two main cell groups (A9 and A10). While there is some overlap in terms of their terminal regions, they are mainly distinguished on the basis of their topographic location (Scheel-Kruger and Willner, 1991). The A9 group arises from the substantia nigra (SN) pars compacta and projects mainly to the caudate-putamen (dorsal striatum) and other areas associated with the basal ganglia. This pathway is referred to as the nigrostriatal DA system. The A10 group corresponds to the ventral tegmental area (VTA) and projects to the NAc, olfactory tubercle, and limbic areas such as the septum, amygdala, and bed nucleus of the stria terminalis. This system is commonly referred to as the mesolimbic DA system. A mesocortical DA system that also originates in the VTA and projects mainly to the prefrontal and cingulate cortices is often grouped with the mesolimbic system, collectively known as the mesocorticolimbic DA system (see Crow and Arbuthnott, 1972).

The principal focus of research on the neuropharmacology of reinforcement has been the origins and terminal areas of the mesocorticolimbic DA system, and there is substantial evidence for the importance of this system in drug reward. Other components include the opioid peptides, γ-aminobutyric acid (GABA), glutamate, serotonin, and presumably other neural inputs that interact with the VTA and the basal forebrain (Koob, 1992). More recent data and observations have also provided support for a functional

neural circuitry within the basal forebrain, termed the extended amygdala (de Olmos and Heimer, 1999). The extended amygdala is composed of several basal forebrain nuclei, including the bed nucleus of the stria terminalis, the centromedial amygdala, and the shell portion of the NAc. These nuclei receive afferent connections from the limbic cortices, the hippocampus, the basolateral amygdala, midbrain, and the lateral hypothalamus. Efferent connections include the medial portion of the ventral pallidum, the VTA, various brain stem projections, and the lateral hypothalamus (Heimer et al., 1991; Zahm and Heimer, 1993). Recent studies have suggested a role of this circuitry in associative processes involved in addiction and reward (Everitt et al., 1999; Koob, 1999).

Since Crow (1973) reported an anatomical correspondence between ICSS sites and DA cells in the SN/VTA, numerous ICSS studies have examined the extent to which the DA system is involved in the reinforcing effects of electrical self-stimulation. Corbett and Wise (1980) implanted moveable electrodes in rats and confirmed that in the diencephalon, the lowest self-stimulation thresholds and the highest response rates were in the areas traversed by the DA fiber bundles. Moreover, in the midbrain, self-stimulation was restricted to the DA-containing cell bodies (A9 and A10) and appeared to be the most robust in areas with the densest packing of DA neurons. Cooper and Breese (1975) found that reducing levels of DA with the selective neurotoxin 6-hydroxydopamine (6-OHDA) produced an acute decrease in self-stimulation response rates in rats. Moreover, treatment with a DA-synthesis inhibitor (α-methyltyrosine) depressed responding in rats with reduced DA levels, but not in control rats. This reduction in ICSS response rates was not observed after treatments that reduced norepinephrine levels. While this implies a role for

DA in ICSS reinforcement, response rates are not a sufficient measurement of ICSS reinforcement efficacy, since DA systems are involved in motor control and movement (Salamone, 1991). An alternative measure assesses the relationship between response rate (y axis) and the pulse frequency or intensity (x axis) of brain stimulation. In this procedure, shifts along the x axis of the rate-frequency or rate-intensity curve indicate changes in reward efficacy, while increases or decreases in the behavioural asymptote indicate changes in performance/motor efficacy (Edmonds and Gallistel, 1974). When Phillips and associates (1989) measured rate-intensity self-stimulation thresholds, they found that self-stimulation via electrode placement in the VTA increased extracellular dopamine release from DA terminal areas (i.e. NAc), suggesting involvement of a dopaminergic substrate in brain stimulation reward.

Support for the role of DA in brain stimulation reinforcement also comes from studies that have examined the effect of DA receptor blockade on ICSS. Systemic administration of the DA receptor antagonist, pimozide, increased reinforcement thresholds without a general disruption of response rate (Zarevics and Setler, 1979), but did not increase MFB-stimulation detection thresholds (Bird and Kornetsky, 1990). Intra-NAc injections of another DA receptor antagonist, α-flupenthixol, also decreased self-stimulation efficacy, indicated by an increase in the reward summation function towards higher values of the number of stimulation pulses (Stellar et al., 1983). In an elegant study, Stellar and Corbett (1989), examined the effects of bilateral administration of cis-flupenthixol into 56 forebrain DA terminal brain areas on MFB rate-frequency ICSS, measured in a runway paradigm. The authors found that cis-flupenthixol disrupted MFB

reinforcement when administered into the NAc, but not when administered into other DA terminal sites, such as the caudate and medial frontal cortex. This implies a role for mesolimbic DA in the reinforcing effects of ICSS, since blockade of DA terminal regions reduced the reinforcing efficacy of ICSS.

Identification of the neural substrate of reinforcement has been greatly facilitated by the use of techniques in which behavioural reinforcement can be obtained, and subsequently altered, through pharmacological activation of reward-relevant pathways. As discussed, pharmacological manipulation of the brain reward system via the administration of receptor antagonists can significantly alter the reinforcing effects of ICSS. Other reinforcers, such as drugs of abuse (e.g., psychostimulants, opiates), also act on the brain stimulation reward system and are reported to decrease ICSS thresholds (Esposito and Kornetsky, 1977; Esposito et al., 1978; Schaefer and Michael, 1988). Such effects on ICSS thresholds have been interpreted as a central sensitization of drugs on the brain reward system.

The fact that drugs of abuse can function as powerful reinforcers on their own has allowed for a more direct approach in the identification of the neural substrates of reward. The behavioural paradigms that have been the most useful in examining the neuropharmacology of reinforcement are the drug self-administration paradigm and the conditioned place paradigm (see van der Kooy, 1987; Phillips and Fibiger, 1987; Carr et al., 1989; Koob and Goeders, 1989; Tzschentke, 1998; Gardner, 2000; Bardo and Bevins, 2000 for reviews). In the drug self-administration paradigm, the animal must attain some predetermined response requirement in order to receive the drug injection. The reward is

given in a response-contingent matter and in most studies, the animal is required to lever-press to obtain a drug injection. In the conditioned place preference (CPP) paradigm, the administration of the drug is independent of the behaviour of the animal and is given in association with a specific environmental stimulus. Here, the animal learns about the relationship between the drug stimulus and environmental stimuli. Whereas the self-administration is based on Skinnerian operant learning, the CPP paradigm is more related to Pavlovian learning and assesses the reinforcing capacity of stimuli by the ability of conditioned stimuli to evoke an approach response.

1.1.1. Psychostimulants

Psychostimulants, such as amphetamine and cocaine, interact with catecholamine-containing neurons and increase levels of DA and norepinephrine by blocking transporters, preventing uptake, or increasing release of these neurotransmitters (Ritz and Kuhar, 1993). Amphetamine and cocaine preferentially stimulate DA release from the NAc (Di Chiara and Imperato, 1988) and psychostimulant abstinence results in a marked reduction of extracellular DA concentrations in the NAc (Rossetti et al., 1992).

Amphetamine (Yokel and Wise, 1978) and cocaine (Deroche et al., 1999) are self-administered by laboratory animals, and under a continuous reinforcement schedule, rats will maintain a stable amount of drug intake that varies inversely with the dose (Koob and Weiss, 1990). Dose-response curves can be obtained with the self-administration paradigm, and these effects lend themselves to pharmacological substitution and

antagonism. Yokel and Wise (1978) found that in rats, intravenous self-administration of amphetamine decreased in a dose-related manner after administration of the DA receptor agonists apomorphine and piribedil. This finding suggests that the DA agonists suppressed amphetamine intake by extending drug satiation within a given inter-response period. Conversely, administration of low doses of DA receptor antagonists, such as pimozide and butaclamol, but not norepinephrine antagonists, increased response rates for intravenous injections of amphetamine (Yokel and Wise, 1975; Yokel and Wise, 1976). Here, administration of DA receptor antagonists mimics the effect of drug dilution, and an increase in response rates is suggested to be a compensation for the reduced reinforcing effects of amphetamine. These findings suggest that DA, but not norepinephrine, is implicated in the reinforcing effects of amphetamine, since a partial blockade of DA receptors produced a partial blockade of the reinforcing effects of amphetamine. Ettenberg and colleagues (1982) found that pretreatment with another DA receptor antagonist α-flupenthixol also produced dose-dependent increases in cocaine self-administration, and others have reported that this increase in response rate is observed with the administration of both DA D_1 and D_2 receptor antagonists (Caine and Koob, 1994).

The role of DA in the reinforcing properties of amphetamine and cocaine has been extended by the findings that 6-OHDA lesions of areas of the mesolimbic DA system disrupt psychostimulant self-administration. Lesions to the NAc disrupted self-administration in rats that were naive to amphetamine self-administration, as well as in rats previously trained to self-administer amphetamine (Lyness et al., 1979b). This

demonstrates a role for DA nerve terminals in the NAc in both the acquisition and maintenance of amphetamine self-administration. 6-OHDA lesions to the NAc also reduce cocaine self-administration (Roberts et al., 1977; Gerrits and van Ree, 1996). This effect is not due to motor deficits, since identical lesions had only a transient effect on food-reinforced operant responding (Roberts et al., 1977). Roberts and Koob (1982) subsequently demonstrated that 6-OHDA lesions to the DA cell bodies in the VTA also reduce cocaine self-administration.

Later studies have examined the role of other mesocorticolimbic brain areas on psychostimulant self-administration. McGregor and associates (1996) trained rats to self-administer cocaine under a progressive ratio (PR) schedule of reinforcement. In a PR schedule, the ratio requirement for obtaining an intravenous injection of drug is systematically increased until the animal ceases to respond (i.e. break point), and the break point has been used as a measure of how much the animal will work for drug intake. The authors found that 6-OHDA lesions to the medial prefrontal cortex caused a significant increase in break point for cocaine self-administration, albeit only at the lower unit doses of cocaine. Moreover, the lesions induced a significant reduction in medial prefrontal cortex DA and its metabolite, 3,4-dihydroxyphenylacetic acid (DOPAC), with no changes in norepinephrine or serotonin levels. These findings suggest that the medial prefrontal cortex is involved in the reinforcing properties of cocaine. Furthermore, it suggests a role for DA in the motivational aspects of reinforcement, since a PR schedule avoids many problems associated with general rates of responding and has been suggested to measure relative strength of reinforcement (Brady and Griffiths, 1976).

The place conditioning paradigm has been extensively used as a measure of reward or aversion. For neuropharmacology studies, place conditioning offers advantages over the self-administration paradigm (Carr et al., 1989). First, drug doses are independent of the animal's behaviour because they are controlled by the experimenter. In contrast, in self-administration studies, the dose administered is dependent on the animal's rate of lever pressing. Second, in CPP paradigm, animals are tested in a drug-free state. This is a major advantage because many drugs produce motor effects, which may obscure measurement of their reinforcing effects and is problematic for interpretation of self-administration data.

Amphetamine and cocaine reliably produce place preferences (Spyraki et al., 1982b; Morency and Beninger, 1986; Hiroi and White, 1991a; Brown and Fibiger, 1993). DA D_1 and D_2 receptor antagonists reliably block amphetamine-induced place preferences (Hoffman and Beninger, 1989; Hiroi and White, 1991a; Bardo et al., 1999), but D_2 receptor antagonists do not appear to block cocaine-induced place preferences (Spyraki et al., 1982a; Mackey and van der Kooy, 1985). Similarly, 6-OHDA lesions of the NAc disrupt the amphetamine-induced CPP (Spyraki et al., 1982b), but seem to have no effect on the cocaine-induced CPP (Spyraki et al., 1982a). The fact that dopaminergic antagonists are effective in blocking cocaine self-administration, but not cocaine-induced place preferences, suggests that the cocaine-induced CPP may be mediated by neurotransmitter systems other than the dopaminergic system. However, a more recent study reported that SCH 23390, but not sulpiride, blocked a cocaine-induced CPP (Cervo and Samanin, 1995). This suggests that the rewarding properties of cocaine in the CPP

task may be more dependent on D_1 than D_2 receptor-mediated mechanisms.

Intracranial self-administration and place conditioning studies are consistent with the NAc as the site mediating the reinforcing effects of amphetamine. Rats will self-administer d-amphetamine directly into the NAc (Hoebel et al., 1983; Phillips et al., 1994). When vehicle was substituted for amphetamine, responding was extinguished and when amphetamine was reinstated, responding on the active lever was resumed (Phillips et al., 1994). Moreover, co-infusion of DA D_1 and D_2 receptor antagonists, either individually or together, enhanced responding on the drug lever, indicating that activation of both DA receptors is required for self-administration of amphetamine into the NAc. Chevrette and colleagues (2002) also reported that rats will self-administer amphetamine into the NAc and, additionally, found that the central nucleus of the amygdala supports amphetamine self-administration in a dose-dependent manner. In contrast to amphetamine, cocaine is not self-administered into the NAc or the VTA, but is self-administered into the medial prefrontal cortex, and this effect is attenuated by co-infusion of the D_2 receptor antagonist sulpiride (Goeders and Smith, 1983). Moreover, response-contingent infusions of cocaine into the medial prefrontal cortex significantly increased DA turnover in the ipsilateral NAc, suggesting that medial prefrontal cortex cocaine self-administration activates DA innervation in the NAc.

Place conditioning findings are similar to self-administration findings in that microinjections of amphetamine, but not cocaine, into the NAc produce a CPP. Carr and White (1986) reported that amphetamine produced a CPP when injected into the NAc, but did not produce a place preference when injected into the medial prefrontal cortex,

striatum, or amygdala. In contrast to the amphetamine CPP, Hemby and associates (1992) did not observe the induction of a place preference following bilateral infusion of cocaine into the NAc. While this finding is consistent with the intracranial cocaine self-administration data, discrepancies between the reinforcing substrates of amphetamine and cocaine may also be related to drug injection sites within the NAc. A more recent study suggests that the NAc appears to be heterogeneous with regard to the acquisition of the psychostimulant-induced CPP (Liao et al., 2000). Microinjections of amphetamine into the core, but not the shell, produced a place preference whereas microinjections of cocaine into the shell, but not the core, produced a CPP. This finding suggests an anatomical dissociation within the NAc for psychostimulant reinforcement, an effect that has been found with other drugs (see Section 1.1.3.2.). Anatomical evidence demonstrates that the shell and core project to different parts of the ventral pallidum (Heimer et al., 1991). Some support for the role of the ventral pallidum in psychostimulant reinforcement comes from findings that bilateral injections of both amphetamine and cocaine into the ventral pallidum induced a place preference (Gong et al., 1996). Furthermore, in a follow-up study, Gong and associates (1997) found that 6-OHDA lesions to the ventral pallidum blocked a place preference induced by a low (5 mg/kg) but not moderate (10 mg/kg) dose of cocaine. Additionally, tissue assays indicated that the lesioned rats had significantly lower DA concentration in the ventral pallidum, but not in the NAc or striatum, and that preference for the cocaine-paired side correlated significantly with DA concentration in the ventral pallidum, but not in the NAc or striatum.

In summary, self-administration and CPP studies indicate that DA in the NAc and

ventral pallidum mediates the reinforcing properties of psychostimulants, although the results are more clear for amphetamine than for cocaine. The other DA terminal site that supports cocaine reinforcement is the medial prefrontal cortex. Moreover, the reinforcing effects of DA within the NAc appear to be mediated by activation of both DA D_1 and D_2 receptors, however, D_1, but not D_2, receptors seem to mediate cocaine-induced place preferences. Little evidence supports a direct role for the amygdala in psychostimulant reinforcement, however, very recent findings indicate otherwise (Chevrette et al., 2002). Finally, while the findings implicate a role for the mesocorticolimbic DA system in the reinforcing effects of psychostimulants, other neurotransmitter systems are likely involved (see Bardo, 1998 for a review).

1.1.2. Opiates

Opioid peptides are distributed throughout the brain and are involved in three major functions: modulation of nociceptive responses to painful stimuli and stressors, homeostatic adaptive functions (e.g. food, water, temperature regulation), and reinforcement (van Ree et al., 1999; Glass et al., 1999; Millan, 2002). Like psychostimulants, opiate drugs, such as morphine and heroin, are readily self-administered by laboratory animals (Weeks, 1962; Harrigan and Downs, 1978; Weeks and Collins, 1978) and increase DA transmission in the NAc (Di Chiara and Imperato, 1986; Wise et al., 1995). The μ-opioid receptor subtype appears to be important for the reinforcing effects of opiates (Negus et al., 1993), and both pure opioid agonists (e.g. morphine and

heroin) and mixed opioid agonists/antagonists serve as positive reinforcers (see van Ree et al., 1999). Systemic and intracerebroventricular (ICV) administration of opioid antagonists, such as naloxone and naltrexone, produce dose-dependent increases in heroin self-administration (Ettenberg et al., 1982; Koob et al., 1984; Vaccarino et al., 1985). This suggests a compensation for the reduced reinforcing effects of heroin. Self-administration of heroin is reported to be disrupted by kainic acid infusions into the NAc, which resulted in destruction of cell bodies yet did not damage catecholamine innervation in areas anterior to the NAc (Zito et al., 1985), but it is unaffected by 6-OHDA lesions to the NAc (Pettit et al., 1984).

Pretreatment with high doses of the DA receptor antagonist, α-flupenthixol, produced a marginal reduction in heroin self-administration, whereas significant increases in cocaine self-administration were observed with similar or lower doses of the antagonist (Ettenberg et al., 1982). This is consistent with the finding that administration of neuroleptics did not affect heroin intake (van Ree and Ramsey, 1987). In contrast, systemic administration of the selective DA D_1 receptor antagonist, SCH 23390, is reported to decrease heroin intake during the initiation of heroin self-administration (Gerrits et al., 1994) and the DA D_2 receptor antagonist, eticlopride, is reported to reduce responding for heroin (Hemby et al., 1996). High doses of these antagonists were used, however, and the decrease in heroin intake may be related to the effects of the antagonists on motor functioning or rate of responding.

Beach (1957) was the first to demonstrate that morphine is reinforcing in terms of place learning. Since then, numerous experiments have demonstrated that opiate drugs

induce a CPP (van der Kooy et al., 1982; Mucha and Iversen, 1984; Iwamoto, 1985b; Bozarth, 1987a), and that administration of opioid antagonists block opiate-induced CPPs (Phillips and LePiane, 1980; Mucha and Iversen, 1984; Piepponen et al., 1997; Olmstead and Franklin, 1997b). As in the case of self-administration data, findings from DA receptor antagonists are inconsistent. Some researchers report that DA receptor antagonists (haloperidol and α-flupenthixol) block morphine-induced place preferences (Acquas et al., 1989; Shippenberg et al., 1993), while others report no effect (Mackey and van der Kooy, 1985). However, D_2 receptor antagonists are reported to block morphine-induced place preferences in opiate-dependent or withdrawn rats, but not drug-naive rats (Nader and van der Kooy, 1997). This suggests that dopaminergic systems may mediate morphine reward in opiate deprivation states.

Kelsey and colleagues (1989) found that while bilateral electrolytic lesions of the NAc abolished a morphine-induced CPP in rats, the lesions did not alter the capacity to establish context-specific tolerance to the analgesic effects of morphine. According to the authors, this suggests that the NAc lesions did not disrupt the ability of the animals to associate morphine with a particular environment, but instead disrupted a pathway that is critical in mediating the reinforcing effects of morphine in the CPP task. However, the authors used a biased CPP paradigm, which confounds the interpretation of the results (see Carr et al., 1989). Furthermore, electrolytic lesions destroy both the cell bodies and fibers that pass through the NAc. Later studies reported that neurotoxin lesions to the NAc, which do not destroy fibers of passage, did not disrupt a morphine-induced CPP (Olmstead and Franklin, 1996; Olmstead and Franklin, 1997a). Moreover, microinjections

of diazepam into the NAc did not prevent acquisition or expression of a morphine-induced CPP (Leri and Franklin, 2000a). Since diazepam and other benzodiazepines reduce DA release from the NAc (Invernizzi et al., 1991; Finlay et al., 1992), and since neurotoxin lesions to the NAc do not disrupt the morphine-induced CPP, the reinforcing effects of morphine in the CPP task are not dependent on stimulation of dopaminergic terminals in the NAc.

Intracranial self-administration and place conditioning studies consistently delineate a role for the VTA in opiate reinforcement. Bozarth and Wise (1981) were the first to demonstrate that rats would self-administer morphine into the VTA on a continuous reinforcement schedule. Response rates for vehicle were much lower, and systemic administration of naloxone reduced responding for morphine to vehicle control levels. In a later study, Devine and Wise (1994) reported that rats self-administered morphine (a mixed μ-opioid and δ-opioid agonist), the selective μ-opioid agonist DAMGO, and the selective δ-opioid agonist DPDPE into the VTA. Moreover, the effective dose for the establishment and maintenance of DAMGO self-administration was 100 times lower than that for morphine or DPDPE, demonstrating that the μ-opioid receptor subtype plays the primary role in the reinforcing effects of opiates in the VTA.

Place conditioning studies also suggest that the VTA mediates the reinforcing effects of morphine. Phillips and LePiane (1980) reported that bilateral injections of 0.2 - 1.0 μg morphine into the VTA induced a CPP, while injections 2.5 mm dorsal to the VTA had no effect. Others also report that intra-VTA morphine within this dose range produces a place preference (Nader and van der Kooy, 1997; Olmstead and Franklin,

1997b). Furthermore, systemic or intra-VTA administration of naloxone blocked an intra-VTA- or systemic-induced morphine CPP, respectively (Phillips and LePiane, 1980; Olmstead and Franklin, 1997b). In a mapping study, Bozarth (1987b) reported that injections along the antero-posterior axis of the VTA induced a place preference, whereas infusions rostral or caudal to the VTA did not. These findings suggest that the anatomical boundaries of the reward-relevant opiate-receptor field within the VTA correspond well with the distribution of the A10 DA-containing cell bodies (Bozarth, 1987b).

In addition to the VTA, the NAc may also mediate the reinforcing effects of opiates. Olds (1982) found that rats will self-administer 0.2 μg morphine into the NAc, but not outside of this region. Responses on the active lever were significantly reduced when naloxone was co-infused with morphine. Self-administration of morphine into the NAc, but not the anterior or posterior caudate putamen, has also been reported in mice (David and Cazala, 2000). Moreover, Goeders and associates (1984) reported that the endogenous opioid methionine enkephalin was also self-administered into the NAc and that this effect was blocked by administration of naloxone.

In terms of place conditioning, van der Kooy and colleagues (1982) found that intra-NAc morphine induced a significant place preference, while Olmstead and Franklin (1997b) did not obtain a CPP when morphine was injected into either the NAc shell or core. Schildein and associates (1998) also did not obtain a significant CPP with unilateral injections of morphine into the NAc, despite finding that the morphine injections stimulated behavioural activity and elicited contraversive turning. It is possible that the studies that did not obtain a significant CPP to intra-NAc morphine used too low doses.

However, microinjections of the μ-opioid agonist DAMGO into the NAc also did not induce a significant CPP (Bals-Kubik et al., 1993).

It is widely accepted that the reinforcing effects of opiates in the VTA are mediated by μ receptors expressed by GABA-containing cells in this region, and that activation of μ receptors inhibit GABAergic cells which normally inhibit dopaminergic cells (Johnson and North, 1992a). However, studies of GABAergic agents have suggested that the anterior and posterior VTA are heterogeneous in terms of reinforcement (see Section 1.1.3.). To test whether the VTA is also heterogeneous in term of opioid reinforcement, a recent study examined whether administration of endomorphin-1, an endogenous opioid peptide selective for the μ receptor, would produce differential reinforcing effects when administered into the anterior or posterior VTA (Zangen et al., 2002). The authors found that rats self-administered endomorphin-1 (50 pmol) into either the anterior or posterior VTA, but not into the NAc. Substitution of vehicle extinguished responding on the active lever, and responding resumed when endomorphin-1 self-administration was re-established. A dose-dependent effect for endomorphin-1 was observed only in the posterior VTA, with a higher dose (250 pmol) producing a greater number of responses and a more regular response rate than the lower dose (50 pmol) or either dose self-infused into the anterior VTA. Furthermore, administration of endomorphin-1 into the posterior VTA produced a significant place preference, but did not produce a CPP when administered into the anterior VTA or subdivisions of the NAc (shell and core). The authors suggest that these findings do not necessarily indicate that there are regional differences for opioid receptor function in the

VTA, since both the anterior and posterior VTA supported endomorphin-1 self-administration. Instead, the more robust effects found in the posterior VTA may be because there is a higher density of opioid receptors within this region (Mansour et al., 1995). Moreover, this is not contradictory to the findings by Bozarth (1987b). While Bozarth did not observe differential reinforcing effects for the intra-VTA morphine-induced CPP, he used relatively higher doses of morphine. Higher doses would spread further and could potentially offer a less sensitive index of anatomic localization.

Other brain sites have also been implicated in the reinforcing effects of opiates. The periaqueductal gray (PAG), a midbrain structure that mediates the analgesic effects of opiates (Yaksh et al., 1976) and relays antinociceptive information from the amygdala to the spinal cord (Oliveira and Prado, 2001), also supports morphine reinforcement. Mice will self-administer morphine into the PAG, and this effect is blocked by administration of naloxone (Cazala, 1990; David and Cazala, 1994b). Administration of methylnaltrexone into the PAG produces dose-related increases in heroin self-administration, and does not significantly alter activity on a control lever or increase responses for cocaine self-administration (Corrigall and Vaccarino, 1988). Furthermore, morphine administered into the PAG induces a significant place preference (van der Kooy et al., 1982; Olmstead and Franklin, 1997b). Areas, such as the lateral hypothalamus, hippocampus, and amygdala have produced inconsistent results. While animals will self-administer morphine into the lateral hypothalamus (Cazala et al., 1987; Cazala, 1990), hippocampus (Self and Stein, 1993), and amygdala (David and Cazala, 1994a), some report place preferences with morphine injections into the lateral hypothalamus (van der Kooy et al., 1982) and

hippocampus (Corrigall and Linseman, 1988), while others do not (Bals-Kubik et al., 1993; Olmstead and Franklin, 1997b).

In summary, the results clearly indicate a reinforcing effect for μ-opioid receptor agonists (e.g. morphine) in the VTA, and this reinforcing effect is blocked by administration of opioid antagonists. The PAG also appears to mediate reinforcing effects of morphine, and support for this comes from both intracranial self-administration and place conditioning studies. Other areas, such as the NAc, lateral hypothalamus, and hippocampus have produced inconsistent findings in terms of their involvement in the rewarding effects of morphine. It has been postulated that opiate actions in the NAc vary as a result of differences in medium spiny neuron circuits (Hakan, 2001), but the mechanism by which opiates are directly reinforcing in the NAc is not known. This along with inconsistent findings in both self-administration and place conditioning paradigms have prompted researchers to propose that opiate reinforcement may depend on motivational states (Laviolette et al., 2002) and involve both a dopamine-dependent (VTA) and dopamine-independent (NAc) component (Koob, 1992; Leri and Franklin, 2000a).

1.1.3. Ethanol and GABAergic agents

Ethanol, barbiturates, and benzodiazepines, belong to the drug class known as sedative-hypnotics, and alter neurotransmission via facilitation of the GABAergic system (Hevers and Luddens, 1998). Considerable research has been devoted to the neurobiology

of alcohol abuse, since reports indicate that alcohol use continues to be a significant threat to world health (World Health Organization, 2001). However, compared to other drugs of abuse, there is relatively less known about the neuropharmacological mechanisms that mediate the reinforcing effects of these central nervous system (CNS) depressants. It has been difficult to draw a clear profile of ethanol reinforcement, since ethanol is believed to produce its reinforcing actions through a number of neurochemical systems. For this reason, ethanol may not be the best candidate by which to examine the neuropharmacological mechanisms of sedative-hypnotic reinforcement. In contrast, the pharmacological characteristics of barbiturates are more clearly defined (see Section 1.2.4.). Moreover, there is an extensive literature on the reinforcing effects of barbiturates in both humans and animals (see Section 1.2.1.). Despite this, there has been no study to date that has examined the neuropharmacological mechanisms involved in their reinforcing properties. This is possibly due to a diminished interest in barbiturate drug effects, since their clinical use has decreased substantially in the last few decades because of the emergence of safer drugs with less abuse potential. While a number of studies have examined the effects of benzodiazepines on the reinforcement system, they are not as readily self-administered as the barbiturates and do not consistently produce place preferences. Because of this, their abuse liability has been questioned by many researchers (see Woods et al., 1987 for a review). Since the barbiturates will be reviewed in Section 1.2., the following section will briefly address the mechanisms involved in ethanol reinforcement. Moreover, because ethanol and barbiturates act on the $GABA_A$ receptor, it is important to first describe some of the effects of $GABA_A$ receptor agents on the

reinforcement system.

1.1.3.1. GABA agents

If, as previously discussed, the reinforcing action of intra-VTA opiates is to inhibit GABA interneurons, thereby disinhibiting DA neurons, then local administration of GABA receptor antagonists should block tonic GABA-mediated inhibition and enhance the activity of VTA DA neurons. David and associates (1997) found that mice self-administered the $GABA_A$ antagonist bicuculline into the VTA in a Y-maze discrimination task. Moreover, systemic administration of the DA receptor antagonist, sulpiride, blocked both the initial acquisition and maintenance of intra-VTA bicuculline self-administration. Consistent with this, other studies report that increasing GABA transmission in the VTA attenuates drug-induced reinforcement. Intra-VTA administration of the $GABA_B$ receptor agonist, baclofen, produced a significant reduction in cocaine-reinforced break points at a dose that was considerably lower than doses necessary to produce comparable reductions in the NAc or striatum (Brebner et al., 2000). Xi and Stein (2000) reported that gamma-vinyl-GABA (GVG), an irreversible GABA-transaminase inhibitor, dose-dependently blocked heroin self-administration (assessed by an increase in heroin self-administration) when administered directly into the lateral ventricles, the VTA, or the ventral pallidum, but not when administered into the NAc. This effect lasted 3-5 days, and prevented or delayed acquisition of heroin self-administration in drug-naive rats. Moreover, the GVG antagonism of the reinforcing effects of heroin was prevented or reversed by systemic or intra-VTA administration of a $GABA_B$ receptor antagonist, but not by administration of

bicuculline. These findings suggest that pharmacological elevation of mesolimbic GABA concentrations reduces opiate reinforcement by activation of $GABA_B$ receptors.

Findings from *in vivo* microdialysis and voltammetry studies support the idea that $GABA_A$ and $GABA_B$ receptors act differently on DA neurons in the VTA. Klitenick and colleagues (1992) reported that administration of morphine through a dialysis probe elicited significant increases in extracellular DA levels and a reduction in GABA levels in the VTA. Administration of baclofen, a $GABA_B$ agonist, produced the opposite effect of morphine on extracellular DA levels in the VTA. In contrast to baclofen administration, application of muscimol, a $GABA_A$ receptor agonist, through the dialysis probe elicited a significant increase in VTA extracellular levels of DA. This suggests that while $GABA_B$ receptor stimulation hyperpolarizes DA neurons, stimulation of $GABA_A$ receptors increases somatodendritic DA release by inhibiting tonic GABAergic input (via GABA interneurons) to DA cells. Further support for this comes from findings that both intravenous and intra-VTA administration of muscimol increased DA release from the NAc, and intra-VTA bicuculline pretreatment blocked this effect (Xi and Stein, 1998). In contrast, intra-VTA injections of baclofen significantly decreased basal DA release in the NAc, and administration of the $GABA_B$ antagonist 2-OH-saclofen into the VTA increased DA release. Moreover, pretreatment with baclofen blocked the muscimol-induced increase in DA.

While this finding suggests that $GABA_A$ and $GABA_B$ receptors have different modulatory effects on the mesolimbic DA system, it does not indicate whether these effects are similar within different parts of the VTA. A recent study found that

administration of both muscimol and bicuculline methiodide (a quaternary derivative of bicuculline which does not cross the blood brain barrier) into the VTA produced rewarding effects, assessed by the ability of both agents to produce a significant place preference (Laviolette and van der Kooy, 2001). Furthermore, the authors found that systemic administration of the DA receptor antagonist α-flupenthixol, or co-administration of baclofen, blocked the reinforcing effects of muscimol, but had no effects on the reinforcing effects of bicuculline. Based on this finding, the authors suggest that there are two populations of $GABA_A$ receptors in the VTA that independently regulate the activity of DA-dependent ($GABA_A$ agonists) and DA-independent ($GABA_A$ antagonists) brain reward systems. While this evidence supports the differential effects of $GABA_A$ and $GABA_B$ receptor activation, the authors did not find a correlation between behavioural effects and injection site in the anterior or the posterior VTA. However, other studies have observed such regional differences. Ikemoto and associates (1997b) demonstrated that rats readily self-administered the $GABA_A$ antagonist, picrotoxin, into the anterior VTA (anterior to −5.2 mm posterior to bregma, according to Paxinos and Watson, 1998), but not into the posterior VTA, SN, or sites dorsal to the VTA. Co-infusion of the $GABA_A$ agonist, muscimol, with picrotoxin significantly reduced self-administration of picrotoxin and substituting bicuculline methiodide for picrotoxin also supported intra-VTA self-administration. Moreover, picrotoxin self-administration into the anterior VTA increased extracellular DA concentrations in the NAc (Ikemoto et al., 1997a). In a follow-up study, Ikemoto and associates (1998) found that muscimol was self-administered into the posterior VTA (−6.3 to −6.8 mm posterior to bregma), but not into

areas anterior to −5.2 mm posterior to bregma. Additionally, co-infusion of picrotoxin with muscimol into the posterior VTA reduced the number of self-infusions. These findings suggest that different $GABA_A$-mediated circuitry may be operating between the anterior and posterior VTA. This is consistent with evidence that microinjections of $GABA_A$ agonists into the posterior, but not anterior, VTA produced an increase in locomotor activity, while microinjections of $GABA_A$ antagonists increased activity when administered into the anterior, but not posterior, VTA (Arnt and Scheel-Kruger, 1979; Wirtshafter and Klitenick, 1989).

Taken together, it appears that there are regional differences within the VTA, and that this can account for the both the differences in $GABA_A$-mediated circuitry and the DA-inhibitory effects of $GABA_B$ neurons. DA cells within the VTA receive GABAergic input from intrinsic interneurons (Klitenick et al., 1992; Churchill et al., 1992) and from descending afferents arising in the NAc and ventral pallidum (Sugita et al., 1992; Johnson and North, 1992b). The innervation from the descending input is to $GABA_B$ receptors while the interneurons stimulate $GABA_A$ receptors. Given this, stimulation of $GABA_B$ receptors would hyperpolarize DA cells and decrease somatodendritic DA release. In the anterior VTA, $GABA_A$ receptors may also tonically inhibit DA neurons, and activation of these $GABA_A$ receptors would also decrease somatodendritic DA release. In contrast, $GABA_A$ receptor activation in the posterior VTA would increase DA release by inhibiting tonic GABAergic input (via GABAergic interneurons) to DA cells (McBride et al., 1999).

1.1.3.2. Ethanol

Like other major drugs of abuse, administration of ethanol stimulates dopamine release from the NAc (Di Chiara and Imperato, 1986; Kohl et al., 1998). Self-administration of ethanol by laboratory animals, however, is not as robust as the psychostimulants and opiates. While some researchers report that rats will initiate intravenous self-administration of ethanol (Smith and Davis, 1974), others report that they will not (Collins et al., 1984). DeNoble and colleagues (1985) reported that rats provided with unlimited access to intravenous doses of ethanol failed to initiate and maintain lever pressing. Moreover, the rats still did not self-administer ethanol when they were initially trained with pentobarbital. Although a history of drug self-administration is not necessary to establish certain drugs as reinforcers (see Collins et al., 1984), Winger and Woods (1973) found that rhesus monkeys would self-administer ethanol only when they were initially trained with drugs, such as cocaine or the short-acting barbiturate methohexital, that are self-administered *de novo*.

To overcome some of these problems, most experiments use the oral route to study ethanol self-administration. Many researchers use strains of rats (e.g. alcohol-preferring rats) that will readily self-administer ethanol (see McBride and Li, 1998). Alternatively, strains of rats that are not selected to prefer alcohol can be trained to orally self-administer ethanol by progressively replacing sucrose or saccharin with ethanol in the drinking solution (i.e. sweet-fading technique). Under these conditions, ethanol self-administration is readily initiated and maintained (Rassnick et al., 1993; Hyytia and Koob, 1995; Heyser et al., 1999).

As previously mentioned, place conditioning studies are used in order to control for some problems that are inherent in self-administration paradigms. Unfortunately, findings from the reinforcing effects of ethanol in the CPP task are not consistent. van der Kooy and colleagues (1983) found that in drug-naive rats, low doses of ethanol (0.1 - 0.8 g/kg) that produced increases in general activity did not induce a place preference or aversion while higher doses (0.8 - 1.0 g/kg) induced a conditioned place aversion. This effect was not specific to route of administration (intravenous or intragastric), rate of infusion, or concentration of ethanol. Using similar doses of ethanol (0.05 - 1.0 g/kg) but with a different route of administration (intraperitoneal), Asin and associates (1985) reported that ethanol is neutral in the CPP paradigm. To test whether the lack of a place preference or the production of place aversion is related to insufficient exposure to ethanol, Bozarth (1990) increased the number of times rats were conditioned to systemically administered ethanol. After receiving a total of 15 daily conditioning trials, 1.0, but not 0.5 g/kg ethanol induced a significant place preference. Other studies also found that experience with ethanol may be a necessary requirement to unmask its reinforcing effects. Moderate (0.7 and 1.5 g/kg), but not low (0.35 g/kg) or high (2.8 g/kg), doses of ethanol induced a significant CPP in ethanol-experienced alcohol-preferring rats (Ciccocioppo et al., 1999). In ethanol-naive alcohol-preferring rats, only the 0.7 g/kg dose of ethanol was reinforcing. Furthermore, strains of rats that are not selected to prefer alcohol displayed a place preference to ethanol (0.5 g/kg) only after prolonged (20 days) exposure to ethanol (Bienkowski et al., 1995). Interestingly, a recent study reported that ethanol administered into the lateral ventricles induced a significant

place preference in rats (Walker and Ettenberg, 2001). This suggests that systemic administration of ethanol may manifest both reinforcing and aversive effects of ethanol, and that central administration may bypass some of those aversive effects. Moreover, ethanol appears to be reinforcing in mice in place conditioning studies (Cunningham and Prather, 1992; Cunningham et al., 1998). This is consistent with the finding that ethanol is intravenously self-administered by both alcohol-preferring and alcohol-avoiding strains of mice, despite strain differences in preference to oral ethanol (Grahame and Cunningham, 1997). Given this, mice may serve as a better rodent model than rats by which to study the neuropharmacology of ethanol reinforcement.

Attempts to elucidate the neurochemical systems that mediate the reinforcing effects of ethanol have been difficult because several systems appear to be involved, including DA, serotonin, opioid, GABA, and the excitatory amino acids (see Di Chiara et al., 1996; Grobin et al., 1998; Koob et al., 1998; Chester and Cunningham, 2002 for reviews). Since ethanol is similar to the barbiturates and benzodiazepines in that its primary mode of action is to facilitate GABAergic transmission via activation of the $GABA_A$ receptor (Hevers and Luddens, 1998 and see Section 1.2.4. for a review on GABA receptor transmission), it is not surprising that the role of the $GABA_A$ receptor in ethanol reinforcement has been investigated. Picrotoxin, a $GABA_A$ receptor antagonist, is reported to decrease operant self-administration of an ethanol/sucrose solution (Petry, 1997). Given that picrotoxin administration did not reduce self-administration of the sucrose-only solution, its effects appear to be selective for ethanol reinforcement. Furthermore, both the partial inverse benzodiazepine agonist, Ro 15-4513, and the

$GABA_A$ receptor agonist, muscimol, also reduced ethanol self-administration. Like picrotoxin, this reduction cannot be attributed to consummatory responses, since responses for sucrose were not reduced. The reason for this general decrease in ethanol self-administration is not clear, but may be because it is sometimes difficult to interpret the effects of antagonists in the self-administration paradigm. Alternatively, it also could be explained by regional differences of GABAergic neurons in the VTA, in that the net effect of GABA on DA cell function is dependent upon the functional balance of both direct inhibition and indirect disinhibition.

Findings from place conditioning studies are also not clear. Chester and Cunningham (1999) reported that pretreatment with the $GABA_A$ antagonists bicuculline and picrotoxin significantly increased the magnitude of the ethanol-induced CPP compared to vehicle-treated controls. This could explain the finding that picrotoxin reduced ethanol self-administration in that picrotoxin substituted for the reinforcing effects of ethanol by blocking $GABA_A$ receptors in the anterior VTA, thereby disinhibiting DA release in the mesolimbic system. However, it is inconsistent with the finding that $GABA_A$ receptor agonists also decrease self-administration. Furthermore, this place preference finding implies that ethanol does not exert its reinforcing effects via activation of the GABAergic system in the CPP paradigm.

The dopaminergic and opioid systems have also been implicated in ethanol reinforcement. The exact mechanism by which ethanol stimulates DA neurons is not certain, but it has been proposed that ethanol decreases the activity of GABAergic neurons in the SN pars reticulata, which might tonically inhibit DA neurons (Mereu and

Gessa, 1985). Systemic pretreatment with a DA receptor antagonist significantly decreased responding for ethanol without affecting responses for water (Rassnick et al., 1992), and DA receptor antagonists are reported to decrease responding on the active lever in an ethanol-reinstatement model of drug-seeking (Liu and Weiss, 2002). However, a specific reduction of the primary reinforcing properties of ethanol by DA receptor antagonists should result in an increase, rather than a decrease, in operant responding for ethanol. Moreover, 6-OHDA lesions to the NAc do not affect ethanol self-administration (Rassnick et al., 1993; Koistinen et al., 2001), and the neuroleptic haloperidol does not affect the acquisition or expression of an ethanol-induced CPP (Cunningham et al., 1992; Risinger et al., 1992). Despite the inability of researchers to demonstrate a direct role for DA in ethanol reinforcement with neurotoxin lesions or pharmacological antagonism, a recent study examined the role of DA in the drug-seeking properties of ethanol. Melendez and colleagues (2002) found that self-administration of ethanol, but not saccharin or water, increased locomotor activity and DA efflux from the NAc during the first 10 minutes of an anticipation period in rats of an alcohol-preferring strain. Both the ethanol and saccharin group showed increased locomotor activity during the first 10 minutes of the self-administration period, but only the ethanol group showed increased DA release during the 20^{th} and 30^{th} minute of the self-administration period and during the first 10 minutes of the post-administration period. According to the authors, this demonstrates that anticipation of self-administered ethanol, as well as operant self-administration of ethanol is associated with increased locomotor activity and DA release from the NAc in this strain of rats, and suggests that such neurochemical correlates are associated with the

development and maintenance of ethanol-seeking behaviour.

The role of opioids in ethanol reinforcement has been studied more extensively, and considerable evidence has implicated the role of the endogenous opioid system in alcohol addiction (see Herz, 1997). Samson and Doyle (1985) reported that pretreatment with 20 mg/kg naloxone, but not 5 or 10 mg/kg naloxone, decreased responding for ethanol in rats, but did not produce effects on water responding. Moreover, naloxone pretreatment did not alter responding for sucrose which indicates that naloxone did not produce a general reduction in consummatory behaviour. However, these results should be interpreted with caution, since the specificity of naloxone as an opioid receptor antagonist decreases as the dose of naloxone increases, and high doses of naloxone may produce effects on other neurotransmitter systems, such as GABA (Sawynok et al., 1979). Using lower doses of the non-selective opioid antagonist, naltrexone, Bienkowski and associates (1999) reported that repeated (3 mg/kg), but not acute (1 - 3 mg/kg), administration of naltrexone decreased ethanol self-administration. Moreover, the authors found that acute administration of the antagonist increased extinction and attenuated cue-induced reinstatement of ethanol-reinforced behaviour. The effects of naltrexone on ethanol reinforcement have also been studied in non-human primates, but findings indicate that these effects are not selective to ethanol reinforcement. Rodefer and colleagues (1999) found that while naltrexone pretreatment had no effect on food- or phencyclidine-maintained responding in rhesus monkeys, it reduced both ethanol- and saccharin-maintained responding. Similarly, Williams and associates (1998) reported that while naltrexone reduced both oral and intravenous ethanol-reinforced responding in monkeys,

assessed by a downward shift in the ethanol concentration-consumption curve, naltrexone pretreatment also reduced reinforced responding for sucrose.

In terms of place conditioning, findings are not consistent. Whereas Biala and Langwinski (1996) found that 1 mg/kg naloxone blocked an ethanol-induced place preference in rats, Cunningham and associates (1995) reported that naloxone (1.5 or 10.0 mg/kg) did not block acquisition of an ethanol-induced place preference in mice. However, the authors report that naloxone (0.15, 1.5, 3.0, or 10.0 mg/kg) administered during the test session facilitated extinction, since the significant ethanol CPP that was observed during the first 10 minutes of testing decreased dose-dependently over time.

While findings from the systemic administration of $GABA_A$, dopamine, and opioid antagonists on ethanol reinforcement are not consistent, intracranial studies have provided a more clear profile for the reinforcing substrates of ethanol. Because extracellular and intracellular studies have demonstrated that ethanol dose-dependently increases the firing rates of DA neurons in the VTA (Brodie et al., 1990; Brodie and Appel, 1998), many studies have examined the reinforcing effects of intra-VTA ethanol administration. Gatto and associates (1994) found that rats of an alcohol-preferring strain self-administered ethanol directly into mid-posterior VTA, but not into areas dorsal to the VTA. These rats discriminated the active lever from the inactive lever, showed extinction responding when vehicle was substituted for ethanol, and reinstated responding on the active lever when ethanol was restored. However, rats of an alcohol non-preferring strain did not self-administer ethanol at any concentration, suggesting that genetic factors may influence the ability of rats to self-administer ethanol into the VTA. Rodd-Henricks and colleagues

(2000) found that rats not selected for alcohol preference initiated and maintained self-infusion of ethanol into the posterior, but not the anterior, VTA. This was not due to a general increase in behavioural activity, since responding was significantly greater on the active lever than on the inactive lever. Since ethanol stimulates $GABA_A$ receptors, this finding may related to differences in $GABA_A$-mediated circuitry between the anterior and posterior VTA. Another possible mechanism involved in ethanol infusion into the posterior VTA is activation of the serotonergic system. Addition of serotonin or serotonin reuptake inhibitors to VTA brain slices is reported to potentiate the increase in firing rates of DA neurons induced by ethanol (Brodie et al., 1995; Trifunovic and Brodie, 1996) and local perfusion of 5-HT_3 antagonists to the VTA prevented the increase in somatodendritic DA release induced by systemic administration of ethanol (Campbell et al., 1996).

Nowak and associates (1998) tested whether the VTA GABAergic system may be involved in ethanol self-administration. They found that microinjections of picrotoxin or bicuculline methiodide into the anterior VTA, but not into regions outside of the VTA, attenuated ethanol intake in alcohol-preferring rats. Saccharin intake was not affected by administration of the antagonists, and co-infusion of picrotoxin and muscimol reversed the attenuating effects of picrotoxin on ethanol intake. This finding suggests that both picrotoxin and bicuculline methiodide are promoting the effects of ethanol, since less ethanol is consumed in the presence of these antagonists. The mechanism by which these $GABA_A$ receptor antagonists reduce ethanol intake may be the result of blocking tonic inhibition mediated by $GABA_A$ receptors in the anterior VTA which, in turn, activates the VTA DA system.

Some studies indicate that ethanol self-administration can be attenuated or blocked by administration of receptor antagonists into other areas of the mesolimbic system. Hyytia and Kiianmaa (2001) reported that intra-amygdala administration of the μ-opioid antagonist, CTOP, as well as intra-amygdala and intra-accumbens administration of the δ-opioid antagonist, naltrindole, suppressed ethanol self-administration in Wistar rats. Consistent with this finding, Heyser and colleagues (1999) reported that administration of the opioid antagonist, methylnaloxonium hydrobromide, into the amygdala or NAc significantly reduced ethanol self-administration. The amygdala appeared to be more sensitive, however, since a lower dose of the opioid antagonist was needed to suppress responding. In terms of the GABAergic system, injections of the competitive $GABA_A$ receptor antagonist SR 95531 into the central nucleus of the amygdala decreased responding for oral ethanol in a two-lever, free-choice task (Hyytia and Koob, 1995). Higher doses of SR 95531 injected into the NAc and the bed nucleus of the stria terminalis also suppressed ethanol intake, however, this reduction was not selective for ethanol.

Czachowski and associates (2001) trained rats to press a lever for a fixed number of responses that resulted in access to a drinking tube containing 10% ethanol for one 20 minute period per day. Microinjections of the DA D_2 antagonist, raclopride, into the NAc delayed the onset of ethanol-seeking (i.e. appetitive responding) at all doses tested and decreased number of responses at the low and high doses. Raclopride had no effect on the rate of responding or on the latency to begin consuming ethanol. This finding indicates that the mesolimbic DA system is involved the drug-seeking, but not drug-taking, aspects of ethanol reinforcement. $GABA_A$ receptors may also be involved in certain aspects of

ethanol intake. Hodge and colleagues (1995) reported that administration of both muscimol and bicuculline into the NAc decreased ethanol-reinforced responding. When the pattern of reduced responses was analyzed, the authors found that muscimol reduced ethanol self-administration by terminating the responding after a shorter delay without changing the local response rate. Bicuculline, on the other hand, not only shortened the termination period, but also decreased the rate of response. According to the authors, this finding indicates that GABAergic transmission in the NAc is involved in the termination, but not the initiation or maintenance, of ethanol self-administration. However, the fact that both muscimol and bicuculline decreased the latency for response termination is puzzling, and may be explained by regional differences within the NAc. A recent study found that activation of $GABA_A$ receptors in the medial shell of the NAc triggered multiple motivated behaviours that were organized along bivalent rostrocaudal gradients (Reynolds and Berridge, 2002). The authors found that muscimol injections into the most rostral sites increased eating, positive hedonic taste enhancement, and induced a CPP. Conversely, injections into the caudal sites decreased eating, produced negative affective reactions to sucrose, and induced a conditioned place aversion. Given this, a more precise effect of $GABA_A$ agonists and antagonists on ethanol self-administration might be attained with more localized injections within the NAc shell.

In summary, the effects of systemically-administered receptor antagonists on ethanol reinforcement are not consistent. This is particularly true for the self-administration paradigm, since increases or decreases in response rates are difficult to interpret. Place conditioning findings are also not clear, however, the recent report of an

ICV ethanol-induced place preference looks promising (Walker and Ettenberg, 2001). In terms of intracranial self-administration studies, the data suggests that the VTA, particularly the posterior VTA, is involved in ethanol reinforcement. Furthermore, both the VTA GABAergic and dopaminergic systems mediate this reinforcement. The evidence also suggests that the opioid system plays a role in ethanol reinforcement. However, findings from the attenuating effects of opioid antagonists on ethanol self-administration do not completely rule out general "malaise". High doses of opioid antagonists, such a naloxone, produce effects on other neurotransmitter systems, and many studies report that the antagonists produced a general suppression in operant behaviour (i.e. decreased responding for sucrose or water as well as ethanol). Furthermore, it appears that while DA may not be necessary for the primary motivational properties of ethanol, it may be essential for the incentive properties (i.e. drug-seeking) of ethanol-conditioned stimuli. Finally, while not discussed, the serotonergic and excitatory amino acid system is also implicated in ethanol reinforcement (see McBride et al., 1993; Trujillo and Akil, 1995).

1.2. Barbiturates

Nor all the drowsy syrups of the world
Shall ever medicine thee to that sweet sleep
Which thou ow'dst yesterday.
— *William Shakespeare*, Othello

Like ethanol, barbiturates are central nervous system (CNS) depressants that belong to the drug class known as sedative-hypnotics. Low doses of barbiturates induce a

state of relaxation and tranquility whereas moderate doses induce a state of "pleasurable intoxication", foster drowsiness, and can induce sleep (Jacobs and Fehr, 1987). At high doses, barbiturates induce anesthesia and a more severe condition of impairment and intoxication similar to that induced by large amounts of alcohol.

Medically, barbiturates were among the drugs most commonly prescribed for the treatment of anxiety, insomnia, and convulsive disorders, such as epilepsy (Wesson and Smith D.E., 1977). Barbiturates have a low therapeutic index, however, and barbiturate use carries a high risk of lethal overdose. The principal toxic effect of barbiturates is respiratory depression, and tolerance to their respiratory-depressant effects develops less rapidly than tolerance to their pleasurable or sleep-inducing effects (Jacobs and Fehr, 1987). Thus, the margin of safety between a lethal dose and an effective dose decreases as the daily dose increases. While barbiturates are still used as anesthetic and anticonvulsant agents (Roberts and Eng-Bourquin, 1995; Russo and Bressolle, 1998), they have been largely replaced by other pharmacologic agents, such as benzodiazepines, that have a higher therapeutic index and a reportedly lower abuse potential (see Woods et al., 1987).

Face validity for the reinforcing effects of barbiturates comes from history. Barbiturates were widely used clinically during the first half of the 20th century, however, they proved to be very dangerous drugs of abuse and their misuse led to social and physical health problems in the United States and elsewhere (e.g. United States Congress and Senate, 1973; Fejer and Smart, 1973; Allgulander, 1986). Prolonged misuse of these drugs leads to the development of tolerance and profound physical dependence that is characterized by a severe, potentially life-threatening abstinence syndrome following

abrupt withdrawal (Wikler, 1968). Indeed, case reports of barbiturate abuse began to appear in the German literature shortly after sodium barbital (Veronal®) was introduced in 1903 (Wesson and Smith D.E., 1977). It was not until the early 1940s, however, that the dependence-producing qualities of barbiturates were recognized (AMA Committee on Alcoholism and Addiction, 1965). Despite this recognition, supply and demand of these drugs continued to rise, and sedative-hypnotic drug use and abuse increased in Europe after World War II, peaking about 1972 (see Allgulander, 1986). The American Medical Association Committee on Alcoholism and Addiction (1965) reported that in 1962, a survey by the Food and Drug Administration indicated that about one million pounds of barbiturates were available in the United States. Over a one-year period, this was enough drug to supply approximately twenty-four 100 mg doses to every man, woman, and child in the country and about 10 years later, the National Commission on Marijuana and Drug Abuse stated that "barbiturate dependence may be the modern equivalent of the hidden opiate dependence of the late 19th century" (United States Congress and Senate, 1973).

Experimental studies confirm the abuse potential of barbiturates and have established barbiturates as powerful reinforcers in both human subjects and laboratory animals. The next sections will review the effects of barbiturates in a number of behavioural paradigms, including drug discrimination, self-administration, conditioned place preference, and ICSS paradigms, and will then discuss the known pharmacological actions of barbiturates.

1.2.1. Drug Discrimination and Self-Administration Paradigms

1.2.1.1. Animal Experiments

When a designated behaviour is reinforced in the presence of a specified environmental event and the rate of occurrence of the behaviour increases in the presence of this event, the event is called a discriminative stimulus. The discriminative stimulus properties of barbiturates have been studied in rats (York, 1978; Mariathasan and Stolerman, 1994), monkeys (Winger and Herling, 1982), and pigeons (Barrett and Witkin, 1976; Herling et al., 1980; McMillan et al., 2001). Such procedures examine whether a drug can be discriminated from a control (vehicle) substance or from other drugs. Thus, drug discrimination paradigms directly measure the similarity or dissimilarity of perceived (interoceptive) drug effects, and it is hypothesized that if drugs are not readily discriminated from each other, they may act similarly at a neuropharmacological level. In general, appropriate doses of different barbiturates (e.g. methohexital, barbital, and phenobarbital), benzodiazepines (e.g. diazepam), and ethanol produce pentobarbital-like discriminative effects in several species, while drugs from other pharmacological classes, such as opiates and psychomotor stimulants, do not (Barry, 1974).

The ability of barbiturates to substitute for ethanol in discrimination paradigms is well demonstrated (York, 1978; Herling et al., 1980; York and Bush, 1982). Hodge and associates (2001a) reported that systemic administration of pentobarbital (1, 3, and 10 mg/kg) substituted fully for the discriminative stimulus effects of systemic administration

of ethanol (1 g/kg) with sucrose-only reinforcement (ED_{50} of 4.7 ± 1.1 mg/kg). Moreover, the addition of ethanol to the sucrose reinforcement shifted the pentobarbital discrimination ED_{50} significantly to the left to a value of 1.28 ± 0.35 mg/kg. In another study, the authors reported that injections of pentobarbital into the NAc substituted dose-dependently for systemic administration of ethanol (Hodge et al., 2001b). The authors suggest that these findings imply that self-administered ethanol (i.e. when added to sucrose) may produce its discriminative stimulus effects via modulation of the $GABA_A$ receptor system, and that stimulation of $GABA_A$ receptors in the NAc may be involved in the discriminative stimulus effects of systemically administered ethanol. While a mechanism for this effect was not proposed, it may be related to the positive motivational effects of GABAergic agents in the rostral portion of the NAc shell (Reynolds and Berridge, 2002). However, apart from this recent report, there is very little evidence to suggest that the reinforcing effects of GABAergic drugs, such as pentobarbital and ethanol, are mediated by $GABA_A$ receptors in the NAc.

Barbiturate self-administration has been demonstrated in a number of laboratory animals, including monkeys (Winger et al., 1975; Lemaire and Meisch, 1984; Vanover et al., 1989), baboons (Griffiths et al., 1981), rats (Pickens et al., 1981; Collins et al., 1984; DeNoble et al., 1985), and mice (Carney et al., 1991). Early studies of self-administration were performed mainly to examine the effects of barbiturates on tolerance and physical dependence (Yanagita and Takahashi, 1970), whereas later studies employed schedules of reinforcement that more closely examined the abuse liability of these drugs. Winger and colleagues (1975) examined several barbiturates with different durations of action to

determine their ability to promote and maintain self-administration behaviour in rhesus monkeys when daily access was limited to 3 hours per day. They found that all of the barbiturates (pentobarbital, amobarbital, thiopental, methohexital, and barbital) increased and maintained lever pressing and the rate of self-injection was inversely related to the amount of drug injected per response. Furthermore, when saline was substituted for drug, the rate of responding increased abruptly on the 1st day following substitution and then dropped below that of drug-reinforced responding within 4 days.

Some studies have examined the effect of drug concentration on performance under fixed ratio (FR) schedules of reinforcement. Pickens and associates (1981) examined the reinforcing effects of methohexital in rats and found an inverse relationship between response rate and injection dose under FR 1 and 5. However, when methohexital was available under FR 10, 15, and 20, a direct positive relationship between response rate and dose was observed. The pattern of methohexital-reinforced responding was characterized by uniform inter-injection intervals, the duration of which was directly related to the size of the injection dose. Similarly, Lemaire and Meisch (1984) report that as the size of FR increased, the concentration of pentobarbital that maintained the highest rate of responding also increased, while drug intake tended to decrease. Taken together, these findings suggest that relative change in number of reinforcers obtained may be more revealing than measures of absolute rate when comparing behaviour maintained by different magnitudes of a reinforcer. The fact that increasing pentobarbital concentrations maintained the highest rates of responding as FR increased indicates that higher concentrations of pentobarbital exhibit greater reinforcing efficacy. Furthermore, these

findings provide further evidence that low self-administration rates at higher drug concentrations do not necessarily indicate low reinforcing efficacy, and animals appear to self-regulate barbiturate intake when daily access to the drug is limited.

1.2.1.2. Human Subjects

In experiments that use human subjects, paradigms that are analogous to animal discrimination tests examine the subjective or "drug liking" effects of drugs of abuse. Griffiths and colleagues (1980) examined the subjective effects of various oral doses of pentobarbital and diazepam in human subjects with documented histories of sedative abuse. They used the 49-item Addiction Research Center Inventory (ARCI) questionnaire that assesses a broad range of physical, cognitive, and subjective effects of drugs. The items are factor-analyzed into five scales that represent the typical effects of various drugs: the Pentobarbital-Chlorpromazine-Alcohol Group (PCAG) which indicates the sedative effects, the Lysergic Acid (LSD) scale which reflects the hallucinogenic effects, the Amphetamine (A) and Benzedrine Group (BG) which indicate stimulant effects, and the Morphine-Benzedrine Group (MBG) which reflects the euphoric effects of drugs. The authors found that pentobarbital produced dose-related (200-900 mg) increases in subjective- and observer-related drug effects with a trend towards increasing PCAG scores. However, the euphoric effects measured by the MBG scale were not significantly increased.

Griffiths and associates (1983) studied the effects of administering moderate to

high doses of diazepam and pentobarbital to subjects with histories of sedative drug abuse and found that both drugs produced similar dose-related effects on psychomotor performance, daytime sleeping, and staff and subject ratings of drug effects. Interestingly, diazepam, but not pentobarbital, produced dose-related decreases in staff ratings of subjects' mood and social interactions and increases in staff ratings of subjects' hostility and unusual behaviour. The changes in mood and behaviour cannot be attributed to the testing of nonequivalent dose levels of the two drugs, since both drugs produced similar dose-related effects on the other measures. The reasons for the discrepancy between pentobarbital and diazepam on mood and behaviour are not clear, but the negative effects of diazepam on mood and behaviour may partially account for the differences in self-administration results between diazepam and pentobarbital (see below).

Measures of drug liking (i.e. asking subjects how much they "like" a drug's effect) have high face validity and provide a reasonable indication of subjects' general attitude about the drug's effects (de Wit and Griffiths, 1991). In subjects with histories of drug abuse, administration of barbiturates consistently produce high ratings in drug liking (Griffiths et al., 1980; Griffiths et al., 1983; McLeod and Griffiths, 1983). More recently, Mintzer and colleagues (1997) compared the behavioural and sedative effects of ethanol and pentobarbital in subjects with histories of drug abuse. Relative to placebo, both ethanol and pentobarbital produced higher participant ratings of drug liking, measured at the time of administration and in a next-day questionnaire. Post hoc tests indicated that the highest dose of pentobarbital produced significantly higher next-day ratings of drug liking than the highest dose of ethanol. Additionally, pentobarbital was more potent than

ethanol in producing effects on participant-rated drug liking than in producing effects on other subjective and behavioural measures. The authors suggest that these results may indicate that pentobarbital has a greater abuse liability than ethanol.

Although the subjective effects of drugs may indicate a potential for abuse, subjects will self-administer doses of drugs that induce subjective effects similar to placebo (Lamb et al., 1991). This demonstrates that there can be a dissociation between the subjective and reinforcing effects of drugs and that drugs should not be considered to be reinforcing *because* they produce euphoric effects. The reinforcing effects of a drug refers to its ability to increase the probability of a behaviour upon which it has been made contingent. Because the behaviour is a direct measure of drug intake and does not presume the circumstances under which the drug is ingested, it is a more reliable behavioural measure of likelihood of abuse. In human subjects, the reinforcing effects of drugs are studied using either self-administration or choice procedures.

Pentobarbital is reported to be readily self-administered by individuals with a history with sedative abuse. In one of the first reports, Bigelow and associates (1976) allowed subjects to self-administer either diazepam (10 mg dose) or pentobarbital (30 mg dose). Subjects were informed of both the drug and dose used, and drug assignment was based upon subjects' reports of their previous sedative abuse. A maximum of 20 doses was available within each session and individual doses were purchased with tokens earned by exercising on a stationary bicycle. Furthermore, the number of tokens required to earn a single dose of drug was varied across the days. The authors found that subjects readily ingested every possible dose when the response requirement was low, but as response

requirement increased, the amount of drug ingested decreased. A study by Pickens and associates (1977), further demonstrated that both female and male subjects would self-administer 50 mg capsules of pentobarbital when allowed a relatively unrestricted schedule of intake. The subjects regulated their daily pentobarbital intake within individually-defined limits, and showed no evidence of drug intoxication or withdrawal. When subjects were subsequently given a choice for dose preference, most individuals showed pentobarbital dose preference in the intermediate range (50 - 150 mg). Curiously, the size of preferred dose was inversely correlated with daily drug intake in that lower doses of pentobarbital were preferred by individuals with a higher daily intake and higher doses were preferred by subjects with a lower daily drug intake.

Although the earlier studies demonstrated pentobarbital self-administration in human subjects, they did not use placebo controls. In a double-blind study, Griffiths and colleagues (1979) examined the self-administration of pentobarbital, diazepam, chlorpromazine, and placebo in volunteers with histories of sedative drug abuse. The subjects were required to ride a stationary bicycle for 25 minutes in order to obtain a dose of drug, with a maximum of 10 ingestions per day. The authors found that chlorpromazine was similar to placebo in that it did not maintain self-administration, whereas diazepam and pentobarbital maintained levels of self-administration above placebo. However, pentobarbital appeared to maintain more regular self-administration than diazepam and the higher dose of pentobarbital (90 mg) was associated with higher levels of self-administration than the higher dose of diazepam. In a later study, Griffiths and associates (1980) employed a choice procedure and found that subjects chose

pentobarbital over diazepam or placebo and, again, subjects generally preferred the higher doses of pentobarbital.

Finally, the reinforcing effects of different doses of pentobarbital (200, 400, or 600 mg) have been assessed using a progressive ratio (PR) procedure (McLeod and Griffiths, 1983). As previously discussed, under a PR schedule of drug reinforcement, a fixed number of responses initially is required for administration of a drug, and this requirement is increased systematically over successive administration of the drug. In the experiment by McLeod and Griffiths (1983), subjects pressed buttons or rode a stationary bicycle, and the amount of work required to earn successive doses increased over successive sessions until subjects did not meet the PR requirements (i.e. break-point). The authors found that higher doses of pentobarbital maintained behaviour at larger PR values than lower values, with one subject pressing the button 90,000 times and another subject riding the stationary bicycle for 6 hours to obtain a single 600 mg dose of pentobarbital. Findings from all of these studies indicate that subjects with histories of sedative abuse will work for doses of pentobarbital. They consistently choose pentobarbital over placebo and other sedative-like drugs. Furthermore, higher doses of pentobarbital are generally associated with higher rates of drug liking (McLeod and Griffiths, 1983; Mintzer et al., 1997), although in some cases subjects chose a particular dose without reporting increased liking of the drug (Griffiths et al., 1980).

Unlike subjects with histories of sedative abuse, non-drug abusing volunteers do not show a clear preference for pentobarbital over placebo. Using a cumulative dosing procedure, de Wit and associates (1989) found that subjects chose pentobarbital (average

total dose was about 132 mg) only slightly more often than placebo (approximately 52% of the choice opportunities) and their subjective ratings of drug liking only marginally exceeded ratings of placebo liking. However, the finding that pentobarbital liking ratings were positively correlated with the number of pentobarbital doses chosen while placebo liking ratings were not predictive of the number of placebo capsules taken suggests that genuine drug effects during sampling influenced subsequent pentobarbital choices. The reason as to why pentobarbital did not produce a greater preference in normal volunteers is not clear. The authors suggest that it is possible that higher doses are needed to induce a more robust drug liking. Indeed, a more recent study reported that 150 and 300 mg pentobarbital produced an increase in Drug Liking and Good Effects (Rush and Ali, 1999). It should be noted that other potent drugs of abuse, such as amphetamine, are not always preferred over placebo in non-drug abusers (de Wit et al., 1986). Moreover, morphine often produces both positive and negative reactions in non-drug abusers, with participants reporting concurrent ratings of "drug liking" with increased ratings of "feeling bad" (Hill and Zacny, 2000; Marsch et al., 2001). This is consistent with animal studies in which doses of drugs that serve as positive reinforcers can also produce a conditioned taste aversion (Wise et al., 1976).

In summary, experimental studies support the idea that barbiturates are potent reinforcers in operant reinforcement paradigms. The effects of barbiturates are perceived to be similar to other sedative-hypnotics, such as ethanol, and barbiturates are positive reinforcers in the self-administration paradigm. A number of barbiturates with different durations of action are self-administered, and both humans and laboratory animals will

work to obtain successive doses of pentobarbital. Moreover, both humans and animals titrate barbiturate intake, and moderate doses appear to be preferred over lower and higher doses.

1.2.2. Conditioned Place Preference Paradigm

Given that barbiturates are readily self-administered by both humans and laboratory animals, and that human subjects reliably choose pentobarbital over benzodiazepines or placebo, it is surprising that barbiturates have not been found to induce a place preference. Instead, the two studies that have directly examined pentobarbital in the CPP test both report it to be aversive rather than reinforcing.

Mucha and Iversen (1984) found that pentobarbital administered subcutaneously produced a significant dose-dependent conditioned place aversion. The rats avoided the compartments paired with 10 and 20 mg/kg pentobarbital, while neither a preference nor an aversion was demonstrated at lower doses (2.5 and 5.0 mg/kg). Cunningham and Prather (1992) reported that shorter conditioning trials produced a stronger ethanol-induced place preference in mice. Given this, Lew and Parker (1998) examined whether the pentobarbital-induced place aversion would be attenuated or even reversed to a place preference with conditioning trials shorter than the 60 minute trials used by Mucha and Iversen (1984). The authors found that intraperitoneal administration of 15 mg/kg pentobarbital produced a conditioned place aversion regardless of conditioning trial duration (5, 15, 30, or 60 minutes). Moreover, because some researchers reported that

ethanol induced a place aversion in drug-naive rats (van der Kooy et al., 1983) but a place preference in drug-experienced rats (Bienkowski et al., 1995), Lew and Parker tested whether pentobarbital-experienced rats would, like ethanol, show a place preference. When the rats were injected daily with 15 mg/kg pentobarbital for 5 or 15 days prior to the regular CPP paradigm, they no longer showed an aversion to the pentobarbital-paired compartment on test day. However, they still did not show a place preference. While the mechanism involved in the attenuation of the place aversion in pentobarbital-experienced rats is not known, the authors suggest that it could be due to either the development of tolerance to the aversive properties of the drug, or the development of sensitization to the reinforcing properties in combination with the aversive properties of pentobarbital.

One study has examined the effects of phenobarbital in the place preference task. Like pentobarbital, phenobarbital is reported to produce a conditioned place aversion (Wilks and File, 1988). However, this finding should be interpreted cautiously. Firstly, the authors used a biased (or unbalanced) paradigm, which reflects relative preferences/aversions rather than absolute ones (see Carr et al., 1989). Secondly, drug and vehicle injections were not counterbalanced across conditioning days. Finally, the rats were placed in the conditioning compartments when phenobarbital was presumed to be significantly anxiolytic (either 8 hours (50 mg/kg) or 1 hour (20 mg/kg) after phenobarbital injection). There is no evidence to support the idea that a drug produces a stronger place preference when the pairing period occurs during its peak effect phase. Instead, it appears that the onset of drug action may be a more important factor (Fudala and Iwamoto, 1986). Furthermore, phenobarbital may not be the best barbiturate to test

in the CPP paradigm, since reports indicate that it is self-administered at much lower rates than other barbiturates, such as pentobarbital and methohexital (Collins et al., 1984).

1.2.3. Intra-Cranial Self-Stimulation Paradigm

As previously mentioned, drugs such as the psychostimulants and opiates increase the response rate (Stein and Ray, 1960; Broekkamp and Phillips, 1979) and decrease the stimulation intensity needed to maintain some fixed behavioural response (i.e. ICSS intensity threshold) (Kornetsky et al., 1979; Schaefer and Michael, 1988). These effects are thought to reflect a change in reinforcement efficacy of brain stimulation as a result of drug-induced facilitation of reward systems (Predy and Kokkindis, 1984). Given this, all reinforcing drugs should induce such reward-facilitation effects. However, the reinforcing effects of the sedative-hypnotics are anomalous in this paradigm. Ethanol is reported to either increase (Carlson and Lydic, 1976) or have no effect (Schaefer and Michael, 1987) on reinforcement thresholds for ICSS in the lateral hypothalamus. Some researchers report that anxiolytics, such as diazepam, decrease ICSS thresholds in a dose-related manner (Carden and Coons, 1990), while others report minimal changes (Borisenko et al., 1996).

The effects of barbiturates on ICSS have also been inconsistent, but to a lesser degree. Pentobarbital is reported to depress self-stimulation behaviour (Olds and Ito, 1973), but this may be more related to its effects on motor output than on the reinforcement system. Other reports indicate that, under appropriate conditions,

pentobarbital can produce moderate increases in ICSS responding in rats (Gerhardt et al., 1982; Herberg and Williams, 1983). Furthermore, Cooper and associates (1969) reported that the combined administration of amphetamine (0.75 mg/kg) and amobarbital (15 mg/kg) increased self-stimulation response rates beyond that observed when amphetamine was administered by itself. Finally, a recent report indicates that like amphetamine, pentobarbital (2.5 - 10 mg/kg) decreases ICSS thresholds (Bossert and Franklin, unpublished data). This effect appears to be due to the reinforcing effects of pentobarbital on the brain reward system, since gabapentin, an anticonvulsant that has GABA agonist activity but very low abuse potential (Letterman and Markowitz, 1999), increased ICSS thresholds (Bossert and Franklin, unpublished data).

As discussed in Section 1.1.3., there is substantial evidence indicating that the mesolimbic DA system is regulated by GABA neurons in the VTA, and the inconsistent effects of barbiturates in the ICSS paradigm may be related to the net effect of direct inhibition and indirect disinhibition within the VTA. However, less experimental attention has been devoted to these drugs of abuse, and pharmacological studies are lacking. Lorens and Sainati (1978) report that ethanol and the benzodiazepine, chlordiazepoxide, facilitate self-stimulation and that this effect is reversed by administration of naloxone. Similarly, Seeger and colleagues (1981) found that at a dose that does not interfere with operant responding, pentobarbital (10 mg/kg) induced a significant decrease in threshold current. Moreover, the pentobarbital-induced decrease in ICSS threshold was reversed by administration of naloxone (2 mg/kg). The findings from both these studies suggest that GABAergic drugs do in fact facilitate brain stimulation reward and that this mechanism

may involve the endogenous peptide system.

In summary, barbiturate reinforcement is well documented in a number of behavioural paradigms. Unlike other drugs of abuse, however, very little is known about the neuropharmacological mechanisms of barbiturate reinforcement. In contrast, the pharmacological actions that mediate the hypnotic- and anesthetic-inducing effects of barbiturates have been studied more extensively, and may provide insight into the mechanisms that mediate the rewarding effects of barbiturates. Therefore, before proceeding to the experimental sections of this dissertation, it is important to first discuss the known pharmacological actions of barbiturates on the GABAergic system.

1.2.4. Pharmacological Characteristics

The primary pharmacological action of barbiturates is to mimic or facilitate neurotransmission in the GABA system (Koltchine et al., 1996). GABA is the major inhibitory neurotransmitter in the CNS and is found in high concentrations in the mammalian brain and spinal cord. In the rat, the colliculi and the diencephalic regions contain the highest levels of GABA, while lower concentrations are found in whole cerebral hemispheres, the pons, and the medulla (Cooper et al., 1996). GABA is synthesized by the α-decarboxylation of L-glutamic acid, an irreversible reaction that is catalyzed by glutamic acid decarboxylase (GAD). GABA metabolism is related to the oxidative metabolism of carbohydrates in the CNS by means of a "shunt" involving (1) its production from glutamate, (2) its transamination with α-oxoglutarate by GABA

(oxoglutarate transaminase, or GABA-T), yielding succinic semialdehyde and regenerating glutamate, and (3) its entry into the Krebs cycle as succinic acid (via the oxidation of succinic semialdehyde) (Cooper et al., 1996). GAD is unique to mammalian organisms and its location primarily in the CNS correlates well with GABA content. GABA-T, on the other hand, has a wide tissue distribution and while GABA cannot be formed to any extent outside of the CNS, exogenous GABA can be rapidly metabolized by both central and peripheral tissue. Both GAD and GABA-T are dependent on the coenzyme pyridoxal phosphate, and epileptiform seizures can be produced by a lack of this coenzyme or by its inactivation (Cooper et al., 1996).

Drugs can alter GABAergic function in a number of ways. For example, drugs can act on GABA interneurons, which indirectly modifies the amount of GABA that interacts with post-synaptic receptors (Cooper et al., 1996). Moreover, drugs may inhibit the enzymes involved in synthesis and degradation (GAD and GABA-T, respectively) of GABA, or may block the neuronal reuptake of GABA (via the GABA transporter). In terms of postsynaptic receptors, three types of GABA receptors have been identified: $GABA_A$, $GABA_B$, and $GABA_C$ (Costa, 1998). The $GABA_A$ receptor is by far the most prevalent of the known receptors and, consequently, the most extensively studied. Much less is known about the relatively novel $GABA_C$ receptor, and will not be discussed further.

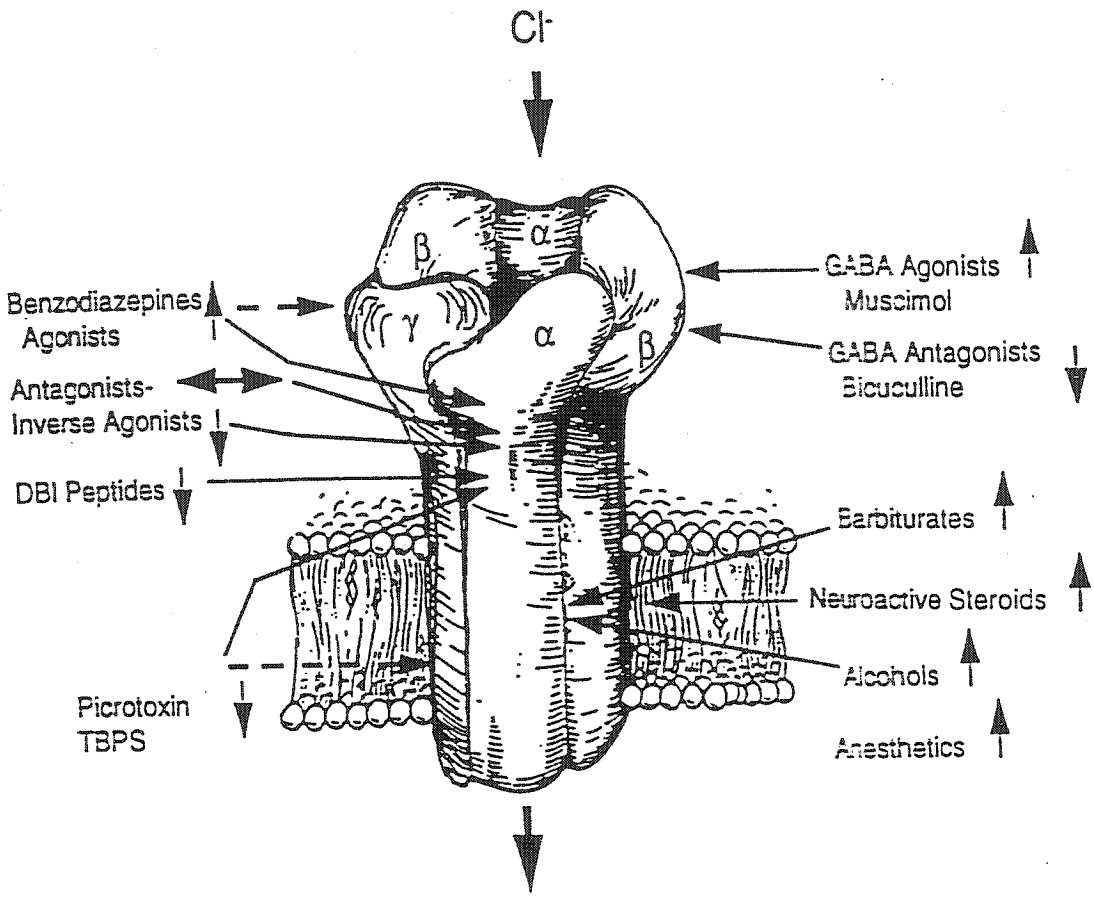
GABA receptors differ in their intracellular mechanisms of transmission: $GABA_A$ receptors are members of the ligand-gated ion channel gene superfamily and are coupled to a chloride (Cl^-) ion channel ($GABA_A$ receptor/chloride ion channel complex) whereas

$GABA_B$ receptors are coupled to calcium or potassium ion channels via GTP-binding proteins (Macdonald and Olsen, 1994). Despite this difference in intracellular mechanisms, binding of GABA to both of these receptors produces hyperpolarization (i.e. GABA is inhibitory). However, GABA can depolarize neurons during embryonic and immediate postnatal corticogenesis, an effect that is believed to be due to reversal of chloride ion gradients (Cherubini et al., 1991). Additionally, excitatory effects of GABA have been recorded in the suprachiasmatic nucleus of the hypothalamus, an area involved in the regulation of circadian rhythms (Wagner et al., 1997). Here, GABA acts as an inhibitory neurotransmitter at night by decreasing the firing frequency. Conversely, during the day, GABA acts as an excitatory neurotransmitter and increases the firing frequency. This effect appears to be due to changes in intracellular Cl^- concentrations, whereby high concentrations prevail during the day and low concentrations prevail at night.

The $GABA_A$ receptors possess a large number of known regulatory sites and pharmacologically relevant ligands which allosterically modulate GABAergic neurotransmission. Such ligands include sedative drugs (barbiturates, benzodiazepines and ethanol), selective agonists (muscimol) and antagonists (bicuculline, SR 95531), the plant convulsant and non-competitive $GABA_A$ receptor antagonist picrotoxin, and the neurosteriods (see Figure 1). Given that barbiturates do not bind to $GABA_B$ receptors, only the $GABA_A$ receptor will be discussed.

The $GABA_A$ receptor is a transmembrane heterooligomeric protein and is believed to be pentameric (Macdonald and Olsen, 1994). It consists of combinations of α, β, γ, δ, and ρ subunit proteins that yield various subtype receptors (e.g. $\alpha 1 \beta 2 \gamma 2$) and at least two

Figure 1. Hypothetical pentameric structure of a $GABA_A$ receptor containing two α and β subunits and a single γ subunit to form a chloride ion (Cl^-) channel. Also shown are many drugs and putative ligands known to interact at one or more sites associated with $GABA_A$ receptors to either positively ($\uparrow$) or negatively ($\downarrow$) modulate GABA-gated Cl^- conductance. Modified from Paul (1995).


Cl-
β
α
γ
α
β
Benzodiazepines
Agonists
Antagonists-
Inverse Agonists
DBI Peptides
Picrotoxin
TBPS
GABA Agonists
Muscimol
GABA Antagonists
Bicuculline
Barbiturates
Neuroactive Steroids
Alcohols
Anesthetics

molecules of GABA must bind to the $GABA_A$ receptor for full activation of the receptor channel. Expression of various combinations of recombinant subunit subtypes in cultured mammalian cell lines suggests that the $GABA_A$ receptor subunit subtype composition is likely responsible for a given pharmacological property (Macdonald and Olsen, 1994). For example, inclusion of a $\gamma 2$ subunit appears to be required for benzodiazepine binding and modulation for $GABA_A$ receptor function (Pritchett et al., 1989; Smith and Olsen, 1995). In contrast to the benzodiazepine site, the barbiturate modulatory site of the $GABA_A$ receptor is less well defined. Sanna and associates (1995) report that direct action of pentobarbital is observed on homomeric $\beta 1$ $GABA_A$ receptors. This suggests that the $\beta 1$ subunits form a functional Cl^- channel that contains sites for direct activation by pentobarbital. However, other studies suggest that both the degree of potentiation of GABA by pentobarbital as well as the degree of affinity and efficacy obtained by the direct activation of pentobarbital on the $GABA_A$ receptor varies with the type of α subunit present (Thompson et al., 1996; Smith et al., 2001). Despite these findings, identification of subunit specificity for barbiturates remains problematic. Some complications include lack of radiolabeled barbiturates with high specific activity, the low affinity of available ligands which may result in non-specific binding, and the lack of a specific barbiturate antagonist (Ito et al., 1996).

The structural diversity of the $GABA_A$ receptor appears to be associated with differences in the channel gating potency of GABA, and this may account for the different behavioural effects of the GABAergic drug classes (Costa, 1998). For example, benzodiazepines increase the frequency of the chloride channel openings without altering

the duration of openings (Haefely, 1987), whereas barbiturates increase the average channel opening duration but do not alter receptor conductance or opening frequency (Macdonald and Olsen, 1994). Like benzodiazepines, barbiturates potentiate GABA-induced increases in Cl^- conductance by increasing the affinity of the $GABA_A$ receptors for GABA. Benzodiazepines, however, have no effect in the absence of GABA (Smith and Olsen, 1995). Barbiturates, on the other hand, have two effects on the $GABA_A$ receptor: low micromolar concentrations potentiate submaximal chloride current responses to GABA whereas higher concentrations cause direct gating of the channel in the absence of GABA (Koltchine et al., 1996). This dual action of barbiturates at the $GABA_A$ receptor is believed to be fundamental to their clinically useful central depressant properties (see Olsen, 1988). More specifically, Schulz and Macdonald (1981) proposed that the potentiation of GABA is relevant to the anticonvulsant properties of barbiturates while the direct GABA-mimetic effects underlie the anesthetic and sedative actions.

In binding studies, barbiturates enhance [^{3}H] GABA and [^{3}H] muscimol binding to rat brain membranes in the presence of chloride (Olsen and Snowman, 1982). They also potentiate the muscimol-mediated suppression of the light-induced increase in dopamine turnover (Kamp and Morgan, 1980). This is consistent with findings by Harrison and Simmonds (1983), who report that barbiturates with a range potencies based on lipid solubility potentiated the effects of muscimol on the $GABA_A$ receptor. The authors also tested the ability of barbiturates to reduce the antagonism of picrotoxin on muscimol superfusion. They found that all of the barbiturates tested reduced the effect of picrotoxin, indicated by a rightward shift in the Schild plot. Interestingly, whereas the

potency of phenobarbital to potentiate muscimol was lower than that of the other barbiturates tested, it was equipotent in its ability to reduce the effects of picrotoxin. This implies that a different site of action is involved in the reduction of the picrotoxin effect. The authors suggest that the ability of phenobarbital to reduce selectively the effects of picrotoxin by an action at a specific site on the $GABA_A$ receptor may be relevant to its anticonvulsant properties. They further suggest that the potentiation of muscimol by barbiturates involves a different site and may underlie the non-anticonvulsant (e.g. hypnotic) properties of these drugs.

The picrotoxin site on the $GABA_A$ receptor was once proposed to be the site of action for barbiturates, since high concentrations of barbiturates inhibit [^{35}S]-*t*-butylbicyclophosphorothionate ([^{35}S] TBPS) binding, which is a typical ligand for the picrotoxin binding site (Wong et al., 1984). Additionally, picrotoxin blocks the barbiturate enhancement of [^{3}H] GABA and [^{3}H] diazepam binding (Leeb-Lundberg et al., 1980; Olsen and Snowman, 1982), whole-cell currents evoked by pentobarbital and GABA (Rho et al., 1996), and pentobarbital-induced blockade of the reduction of pain in the inter-phase of the formalin pain test (Franklin and Abbott, 1993). However, picrotoxin does not block all barbiturate-induced effects. For example, Joy and Albertson (1991) report that whereas bicuculline, a competitive $GABA_A$ antagonist, blocked pentobarbital-enhanced inhibition in the dentate gyrus, picrotoxin did not block this inhibition. Other researchers report that bicuculline, but not picrotoxin, reversed the anticonvulsant effects of pentobarbital on maximal electroshock-induced seizures in rats (Rastogi and Ticku, 1985; Mehta and Ticku, 1986). Furthermore, while barbiturates

markedly accelerate the dissociation of [^{35}S]TBPS binding from its recognition sites, picrotoxin does not affect this dissociation (Trifiletti et al., 1984). Because of this, it is now believed that the barbiturate site and picrotoxin site are distinct, but allosterically coupled.

$GABA_A$ receptor antagonists other than picrotoxin are reported to block barbiturate-induced effects. The competitive antagonists bicuculline and SR 95531 (gabazine) allosterically inhibit pentobarbital-induced channel activation (Ueno et al., 1997) and both antagonists significantly inhibit pentobarbital-stimulated chloride uptake in synaptoneurosomes (Yu and Ho, 1990). Furthermore, barbiturates inhibit the binding of [^{3}H]-bicuculline methochloride and this inhibition is reversed to control levels by 3μm bicuculline (Wong et al., 1984). The allosteric interactions of barbiturates and $GABA_A$ receptor antagonists *in vitro* suggests that such allosteric interactions occur also *in vivo*, and the relative capacity of the different receptor antagonists to inhibit barbiturates is likely related to the specific behavioural effects (i.e. sedative, hypnotic, anticonvulsant) of the barbiturates.

Several studies have shown that barbiturates affect a variety of other ligand-gated and voltage-dependent ion channels. Barbiturates inhibit displaceable [^{14}C]-amobarbitone binding to the *Torpedo* acetylcholine-receptor rich membranes (Dodson et al., 1990), inhibit native nicotinic acetylcholine receptor-mediated currents in rat medial habenula neurons (Kamiya et al., 2001), and appear to be selective antagonists at A_1 adenosine receptors (Lohse et al., 1985). Whereas the action of barbiturates on acetylcholine receptors does not appear to underlie their anesthetic effects (Dodson et al., 1990),

differences among the pharmacological properties of structurally similar barbiturates may be related to their ability to inhibit voltage-dependent calcium channels. ffrench-Mullen and colleagues (1993) reported that the sedative-anesthetic barbiturate, pentobarbital, produced a potent stereoselective blockade of voltage-activated Ca^{2+} channel currents in hippocampal neurons at concentrations that potentiate GABA responses. In contrast, the anticonvulsant barbiturate phenobarbital was considerably weaker as a Ca^{2+} channel blocker than as a potentiator of GABA responses.

There has been some speculation as to whether barbiturates induce anesthesia via suppression of the excitatory amino acid (EAA) neurotransmitter system. Glutamate increases CNS excitability by binding to EAA receptors, resulting in depolarization (Cooper et al., 1996). EAA receptor agonists AMPA (alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) and NMDA (N-methyl-*D*-aspartate) bind to their respective receptors and can induce convulsions (Arnt et al., 1995). Non-competitive NMDA receptor antagonists produce anesthesia (Kubota et al., 1999) and subanesthetic doses of these receptor antagonists potentiate the duration of anesthesia produced by pentobarbital (Daniell, 1990). It is well known that the barbiturate-like sedative, ethanol, blocks NMDA receptors in addition to its inhibitory actions via $GABA_A$ receptors (Simson et al., 1991), so it is possible that the anesthetic effects of barbiturates may be partially mediated via antagonism of the EAA system. However, reports indicate that barbiturate effects on the EAA system are indirect. Kamiya and associates (1999) report that thiopental suppressed the AMPA receptor-mediated current at doses approximately equivalent to those used clinically. However, because both depressant and convulsant barbiturate stereoisomers

also inhibited AMPA receptor-mediated currents, the authors suggest that AMPA receptor inhibition is not important for the hypnotic or anticonvulsant effects of barbiturates. Likewise, the ability of thiopental to inhibit glutamate release was reversed by bicuculline, suggesting that this inhibition of glutamate release is mediated indirectly through actions at the $GABA_A$ receptor (Buggy et al., 2000).

In summary, there is substantial pharmacological evidence that the sedative, hypnotic, and anesthetic effects of barbiturates depend on positive modulation of the $GABA_A$ receptor. As discussed above, barbiturates have an extensive history of abuse and of reinforcing effects that have been demonstrated in human and laboratory animal studies. Given this, it is surprising that so little is known about the neuropharmacological properties of barbiturates and the role of the $GABA_A$ receptor in barbiturate reinforcement. In fact, only one study was found that investigated the pharmacological mechanisms of barbiturate reinforcement. This study demonstrated that naloxone reversed pentobarbital-induced reward-related shifts in ICSS thresholds, and was published over 20 years ago (Seeger et al., 1981). Indeed, compared to other drugs of abuse, there have been fewer experimental investigations devoted to barbiturate reinforcement. Rather, ethanol has been the prototype drug for sedative-hypnotics and has received much more experimental attention than the barbiturates. However, as discussed above, ethanol is not an ideal agent with which to examine the neuropharmacology of reinforcement because of its complex actions on numerous neurochemical systems.

Given the extensive behavioural literature on barbiturate reinforcement, the reported findings that pentobarbital produced a conditioned place aversion are surprising.

Chapter 2 re-examines this issue and shows that pentobarbital will induce a CPP in an unbiased paradigm at doses that are similar to those that produce reward-related shifts in ICSS thresholds (Bossert and Franklin, unpublished data; Seeger et al., 1981). Succeeding chapters exploit the CPP paradigm's advantages for pharmacological investigation to examine the neuropharmacology of barbiturate reinforcement. Chapter 3 examines which neurotransmitter systems may mediate the reinforcing effects of pentobarbital in the CPP task. Chapter 4 examines the reinforcing effects of barbiturates when centrally administered. Chapters 5 and 6 explore the neural substrates and neurochemical systems that mediate those central reinforcing effects. The findings from these experiments are brought together in Chapter 7, and an integrated view of the neuropharmacological mechanisms of barbiturate reinforcement is put forward.

CHAPTER 2:

SODIUM PENTOBARBITAL-INDUCED PLACE PREFERENCE

Why pentobarbital produced a conditioned place aversion in previous studies (Mucha and Iverson, 1984; Lew and Parker, 1998) is not clear. However, there are several aspects of the CPP procedure that could contribute to detecting drug reinforcement in the place preference paradigm. These include timing of drug injection (Fudala and Iwamoto, 1986), route of drug administration (Mayer and Parker, 1993), and number of conditioning trials (Mucha and Iverson, 1984). Given this, the putative reinforcing effects of pentobarbital were re-examined using a paradigm and place preference procedure that has been found to be sensitive to other drugs of abuse (Carr and White, 1983; Olmstead and Franklin, 1997b; Leri and Franklin, 2000c). This procedure used 6 conditioning trials (3 drug-paired and 3 vehicle-paired) in a 3-compartment apparatus and an unbiased CPP paradigm.

2.1. METHODS

For all experiments, animal housing, handling, injections, surgery, and testing procedures were approved by the McGill University Animal Care Committee and conformed to the guidelines of the Canadian Council on Animal Care.

Animals

Subjects were adult male Long Evans rats (Charles River, St. Constance, Quebec), weighing between 150 and 200 grams at the beginning of the experiment. They were housed two or three per cage in a colony room, maintained on a 12 hour light:dark cycle

(lights on at 7am) with constant temperature of approximately 21°C, and had food and water available *ad libitum*. Testing occurred during the light phase at approximately the same time of the day across all of the experiments.

Apparatus

The CPP apparatus consisted of three compartments made of wood, with a plexiglass front wall. Compartments A and B were identical in size (45 X 45 X 30 cm) but differed in shading: The floor of one compartment was painted white and its ceiling was painted black, with black and white vertical stripes on the walls; the floor and ceiling of the other compartment were painted black with black and white horizontal stripes on the walls. Compartment C (36 X 18 X 20 cm) was painted gray and was attached to the rear of compartments A and B. It had removable wooden partitions that separated it from compartments A and B. When the partitions were in place, the rat was confined to one of the larger compartments. When the partitions were removed, the rat could freely move between the two compartments via compartment C. Infrared motion detectors (Model 49-208, Radio Shack) on the back wall of compartment A and B, and light beam sensors on the entrance to compartments C were used to locate the animal. From the signals evoked by the rat's movement, a computer recorded the time spent and locomotor activity in each compartment.

Place Conditioning Procedure

Behavioural testing and conditioning began after 4-6 daily handling sessions. The procedure was divided into three consecutive phases.

Pre-exposure. On the first day of the experiment, the rats were placed in compartment C and the partitions were removed. The rats were allowed to move freely throughout the apparatus for 20 minutes. Time spent and locomotor activity in each compartment were recorded. This phase of testing was performed in all experiments to allow habituation to the apparatus, and to verify that the rats did not exhibit any spontaneous preference for a given compartment.

Conditioning. During this time the partitions between the compartments were in place. This phase lasted a total of 6 days, and consisted of 3 exposures to drug in one compartment and 3 exposures to vehicle in the other compartment. On each training day, the rat was brought to the test room, injected with the drug (or vehicle), and confined to either compartment A or B for 30 minutes. On alternate days, the rat was injected with the vehicle (or drug), and was confined for 30 minutes in the other compartment. Confinement to compartments occurred immediately after injections. The order of injection (drug or vehicle) and the compartment paired with the drug (black or white) was counterbalanced within each group.

Test Phase. 24 h after the last conditioning day, each rat was placed in compartment C with the partitions removed, and was allowed to move freely throughout the apparatus for 20 minutes. Behaviour was recorded as during the pre-exposure phase. The rats did not receive injections of either drug or vehicle during this test phase.

Statistical Analysis

In a balanced (i.e. unbiased) CPP experiment that tests a dose-response relationship, there are two key hypotheses. First, there should be no preference for one side over the other when the animals receive vehicle injections (during conditioning) in both compartments. Second, active drug conditioning injections should induce animals to spend more time on the drug-paired side (compartment) at one or more dose levels of the conditioning drug. To test these hypotheses, the main effects and interaction are not very informative. Instead, the critical statistics are the within-group paired comparisons. These are also the comparisons with the highest power (Hays, 1965), and this is an important consideration with a barbiturate place preference where the effect may be small. Additional hypotheses that could be tested are that the size of the preference is greater at one or more drug doses than at other doses. However, in a mixed model ANOVA, these comparisons have less power (see discussion in Section 3.3). Furthermore, as the number of contrasts tested increases, the probability of a Type 1 error also increases. On the other hand, lowering α to protect the experiment-wise error rate reduces the power of each comparison. To maximize power, it was decided to use planned comparisons and to limit the number of contrasts tested to the within-group comparisons. To account for the experiment-wise error, a Bonferroni correction for α was used, and α was set to 0.05. Because the previous studies (Mucha and Iverson, 1984; Lew and Parker, 1998) report that pentobarbital produced a place aversion, α was two-tailed for the pentobarbital CPP in this chapter. However, for all subsequent experiments in Chapters 3 through 6, the hypotheses are directional, and α was, therefore, one-tailed.

Since the overall ANOVA was not used for hypothesis testing, these statistics are not given in the text, but are listed in Appendix 1. Similar results for locomotor activity in the compartments are given in Appendix 2.

Pentobarbital Conditioned Place Preference

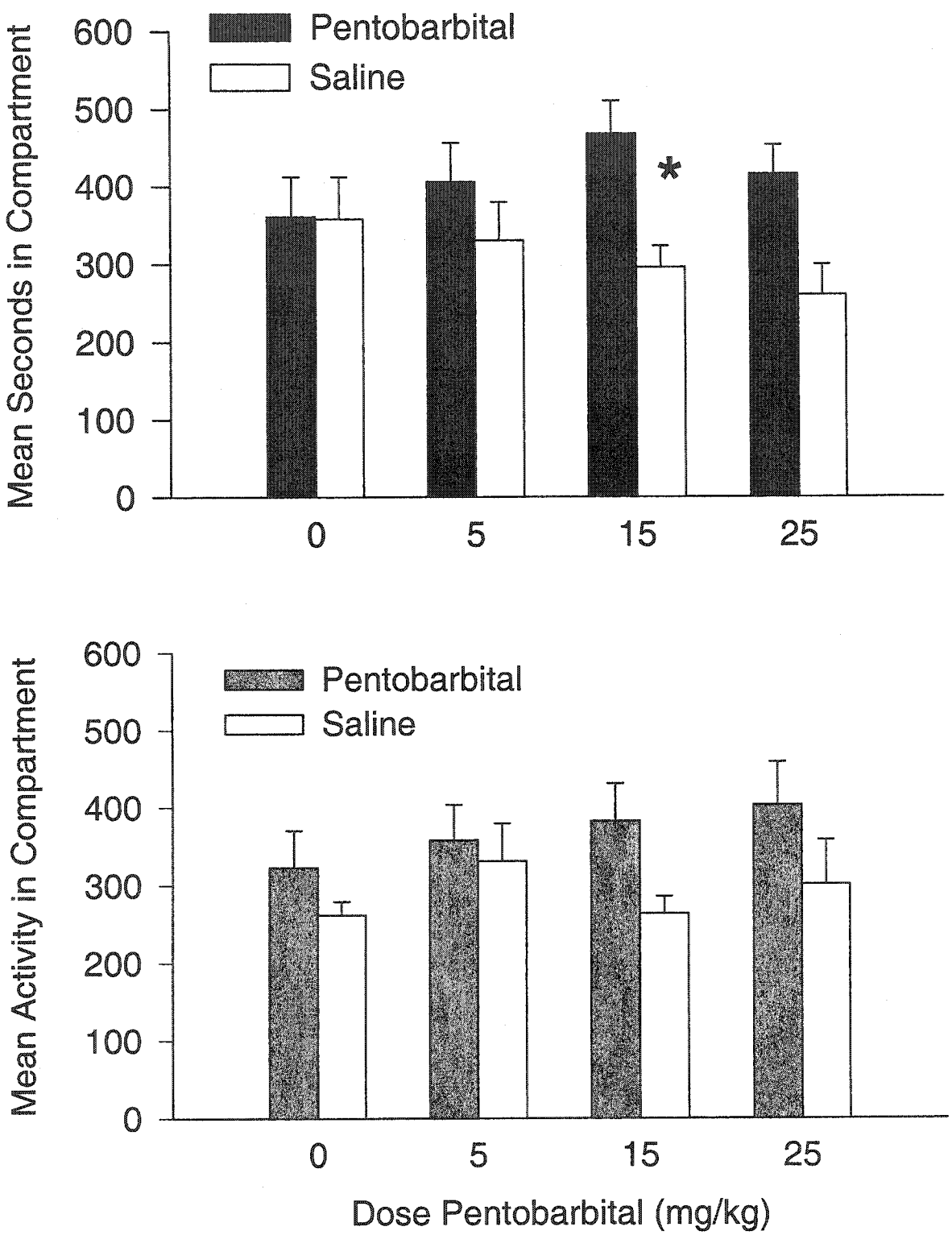
Thirty-two rats were randomly assigned to a control group (0 mg/kg) or one of three dose groups of pentobarbital: 5, 15, or 25 mg/kg (n = 8 for each group). Sodium pentobarbital (65 mg/kg, Somnotol®, MTC Pharmaceuticals, Cambridge, Ontario) was diluted with 0.9 % sodium chloride to 5, 15, and 25 mg/ml and injected intraperitoneally (IP). 0.9 % sodium chloride was injected IP during drug free conditioning trials.

2.2. RESULTS

Planned comparisons revealed that the rats spent significantly more time in the compartment paired with 15 mg/kg pentobarbital than in the compartment paired with saline ($F_{(1,28)} = 4.73$, $p < 0.05$; Figure 2, upper panel). There was a trend for 25 mg/kg ($F_{(1,28)} = 3.87$, $0.05 < p < 0.06$) and no significant difference for 5 mg/kg.

None of the planned comparisons for the different dose groups revealed a significant effect of locomotor activity (Figure 2, lower panel).

Figure 2. Upper panel: Mean time spent (seconds) in compartment paired with 0, 5, 15, and 25 mg/kg pentobarbital (black bars) or saline (white bars). Lower panel: Mean activity counts in compartment paired with 0, 5, 15, and 25 mg/kg pentobarbital (gray bars) or saline (white bars). *Vertical lines* mark the standard error of means (SEM). *Asterisks* indicate a significant difference ($p < 0.05$) between drug and saline condition.


Mean Seconds in Compartment
600
500
400
300
200
100
0
Pentobarbital
Saline
*
0
5
15
25
Mean Activity in Compartment
600
500
400
300
200
100
0
Pentobarbital
Saline
0
5
15
25
Dose Pentobarbital (mg/kg)

2.3. DISCUSSION

The rats spent more time in the pentobarbital-paired compartment than in the saline-paired compartment at all doses tested, but the preference was significant only for 15 mg/kg pentobarbital. There were no significant differences between doses. Moreover, the rats were more active in the pentobarbital-paired compartments, although this increase in activity did not reach statistical significance. It was once thought that there is a common brain circuitry underlying both drug reward and locomotor stimulation (Wise and Bozarth, 1987) and that the unconditioned increase in activity induced by a drug may be causally linked to the production of a CPP and a decrease in activity may be causally linked to the production of a CPA (Swerdlow and Koob, 1984). However, these and other findings suggest that there is not a clear relationship between locomotor activity and place preference. Drugs that produce an increase in activity, such as phencyclidine, have been reported to produce a conditioned place aversion (CPA) (Iwamoto, 1985a) and doses of the local anaesthetic procaine that did not affect locomotor activity still resulted in a significant CPP (Spyraki et al., 1982a). Furthermore, Shimosato and Ohkuma (2000) demonstrated that locomotor sensitization and development of a psychostimulant-induced CPP are dissociable effects. Such findings suggest that while an animal's preference for an environment and its locomotor activity in that environment can be related, animals do not learn to prefer an environment *because* they had previously experienced high levels of locomotor activity in that environment.

While the finding of a pentobarbital-induced place preference is consistent with

animal and human self-administration experiments (Griffiths et al., 1979; Ator and Griffiths, 1987) and human choice preference tests (Griffiths et al., 1980), they are inconsistent with earlier studies that examined the effects of pentobarbital in the CPP paradigm. It is possible that differences between the CPP apparatus and procedure could account for the pentobarbital place preference obtained in this experiment. Both Mucha and Iversen (1984) and Lew and Parker (1998) used a two chamber apparatus, whereas a 3-compartment apparatus was used in this experiment. The use of a 3-compartment apparatus allows for the chambers to be more distinct from each other, since the compartments are completely separate and the cues in one cannot be seen from the other during either the pre-exposure or test sessions. Procedural differences may also contribute to the discrepant findings. Like Lew and Parker (1998), this experiment administered pentobarbital intraperitoneally (IP). However, Lew and Parker placed the rats in the conditioning chambers 5 minutes after the injection, whereas the rats in this experiment were placed into the compartments immediately after injection. Because pentobarbital is lipid soluble (Ho et al., 1975) and rapidly absorbed from the peritoneal cavity, it is possible that the onset of the reinforcing effect of the drug is more closely associated with the apparatus cues in this experiment. Although, Mucha and Iversen (1984) placed the rats in the apparatus immediately after injection, they administered pentobarbital subcutaneously (SC), which leads to slow absorption. Again, it is possible that the onset of the reinforcing effect of the drug is more closely associated with the apparatus cues in the present experiment. Moreover, Mayer and Parker (1993) reported that IP, but not SC, cocaine produces a place preference. This suggests that route of administration,

which is related to the pharmacokinetics of drugs, may be a critical variable for the induction of a place preference of some drugs. Lastly, a total of 6 conditioning trials (3 drug-paired and 3 vehicle-paired) were used in this experiment, whereas Lew and Parker used a total of 4 conditioning trials. While some drugs, such as morphine, produce a CPP with only one conditioning trial (Mucha et al., 1982), Mucha and Iversen (1984) demonstrated that when 1, 2, 3, or 4 pairings were tested, the magnitude of the morphine-induced CPP increased with additional pairings.

The place preference paradigm as used here can demonstrate the reinforcing effects of pentobarbital predicted by animal and human self-administration studies (see Section 1.2.1.). Therefore, it is possible to exploit the advantages of the CPP paradigm to investigate the neuropharmacological basis of pentobarbital reinforcement. Chapter 3 examines the effects of systemic pretreatment with $GABA_A$ (bicuculline or picrotoxin), DA (eticlopride), or opioid (naloxone) receptor antagonists on the pentobarbital-induced CPP.

CHAPTER THREE:

EFFECTS OF PERIPHERAL ANTAGONISTS ON THE PENTOBARBITAL PLACE PREFERENCE

Since the primary pharmacological action of barbiturates is to facilitate neurotransmission in the GABA system (Koltchine et al., 1996), the reinforcing effects of barbiturates might be expected to be mediated by activity at the $GABA_A$ receptor. Moreover, pentobarbital and ethanol have common pharmacological actions, and ethanol self-administration is reported to be mediated by $GABA_A$ receptors in the VTA (Nowak et al., 1998). To test this hypothesis, the effects of systemic pretreatment of the $GABA_A$ receptor antagonists, bicuculline or picrotoxin, on the pentobarbital-induced place preference were examined.

As discussed above in Chapter 1, the reinforcing properties of the major drugs of abuse are thought to be mediated by the mesolimbic DA system. The reinforcing effects of barbiturates may likewise be mediated by the dopaminergic system. Therefore, the effects of systemic pretreatment with the DA D_2 receptor antagonist, eticlopride, on the pentobarbital-induced CPP were also examined.

The μ opioid system is also implicated in drug reinforcement. The opioid antagonist naloxone is reported to block place preferences produced by morphine (Phillips and LePiane, 1980), psychostimulants (Trujillo et al., 1991; Kim et al., 1997), water (Agmo et al., 1993), and sucrose (Agmo et al., 1995). Moreover, because naloxone is reported to block the self-stimulation facilitating effects of ethanol (Lorens and Sainati, 1978) and block pentobarbital-induced reward-related decreases in ICSS threshold (Seeger et al., 1981), it is likely that opioids also mediate barbiturate reinforcement. To test this, the effects of systemic pretreatment with the opioid antagonist, naloxone, on the pentobarbital-induced CPP were also examined.

3.1. METHODS

Animals

Subjects were 200 adult male Long Evans rats. Weight of rats and housing conditions were the same as in Section 2.1.

Apparatus

See Section 2.1.

Place Conditioning Procedure

The CPP procedure was the same as in Section 2.1. For antagonist drug pretreatment, the rats were injected in the colony room and then transported to the test room, with the exception of naloxone which was injected outside of the test room.

Statistical Analysis

See Section 2.1. For samples that resulted in heterogenous error variances (significant values for Levene's test of equality of error variances), logarithmic transformations were computed.

3.1.1. Picrotoxin or Bicuculline Pretreatment to Pentobarbital CPP

During conditioning, the rats were pretreated with picrotoxin (0.5, 1.0, and 2.0 mg/kg; n = 8 per group) or bicuculline (0.5, 1.0, and 2.0 mg/kg; n = 8 per group) or the

appropriate vehicle 25 minutes before pentobarbital (15 mg/kg) treatment. Vehicle injections were also given 25 minutes prior to saline-paired trials. Pentobarbital was diluted and injected as in Section 2.1. Both picrotoxin and bicuculline were prepared fresh daily, and were injected IP in a volume of 1 ml/kg. Picrotoxin (Sigma) was dissolved in saline to obtain the different doses. Bicuculline (Sigma) was dissolved in 0.1M PBS (pH = 5.5) and kept on ice. Additionally, some rats pretreated with picrotoxin (2.0 mg/kg; n = 8) or bicuculline (2.0 mg/kg; n = 8) received only saline on conditioning trials to test for potential aversive effects of these drugs.

3.1.2. Eticlopride Pretreatment to Amphetamine or Pentobarbital CPP

Rats were injected subcutaneously (SC) with eticlopride (0.01, 0.05, and 0.25 mg/kg; n = 8 per group) or the appropriate vehicle 60 minutes prior to *d*-amphetamine (1 mg/kg) or pentobarbital (15 mg/kg) conditioning. Eticlopride (Sigma) was dissolved in saline and injected in a volume of 1 ml/kg.

When an aversive antagonist drug is given together with a reinforcing drug on only one side of a CPP apparatus, there is a possibility that an apparent block of the CPP is due to the summation of independent aversive and reinforcing effects of the two drugs. On the other hand, if an aversive antagonist is given on both sides, a false CPP may be observed because the agonist drug blocks the aversive consequences of antagonist administration. Because many drugs have some aversive consequences, it is typical to administer the antagonist only with the agonist. However, since eticlopride and other dopamine antagonists do not produce aversive effects when administered alone (Hoffman and

Beninger, 1989; Hoffman, 1994), eticlopride injections were given on both drug- and saline-paired trials in this experiment.

3.1.3. Naloxone Pretreatment to Pentobarbital CPP

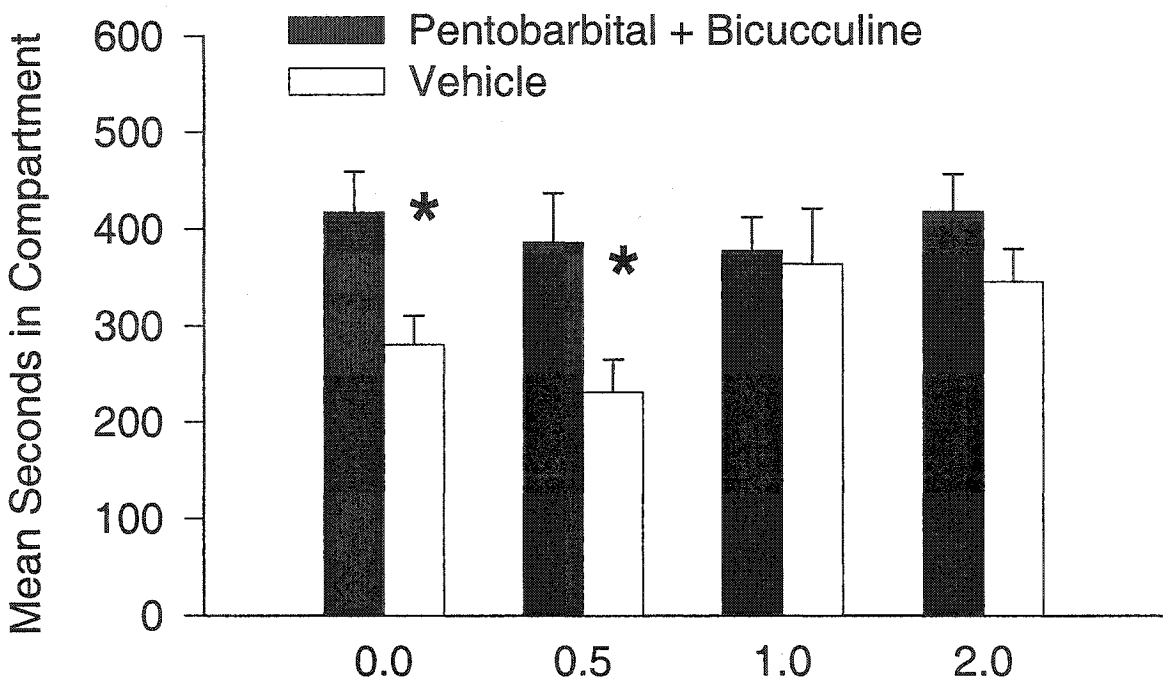
Rats were pretreated with naloxone (0.02, 0.2, and 2.0 mg/kg; n = 8 per group) 5 minutes before pentobarbital (15 mg/kg) conditioning. Naloxone (Sigma) was dissolved in dH_20 and injected IP in a volume of 1 ml/kg. Injections of naloxone occurred only on drug-paired trials and vehicle injections were given 5 minutes prior to saline-paired trials. In addition, some rats pretreated with naloxone (0.02, 0.2, and 2.0 mg/kg; n = 8 per group) received only saline on all conditioning trials to test for potential aversive effects of naloxone.

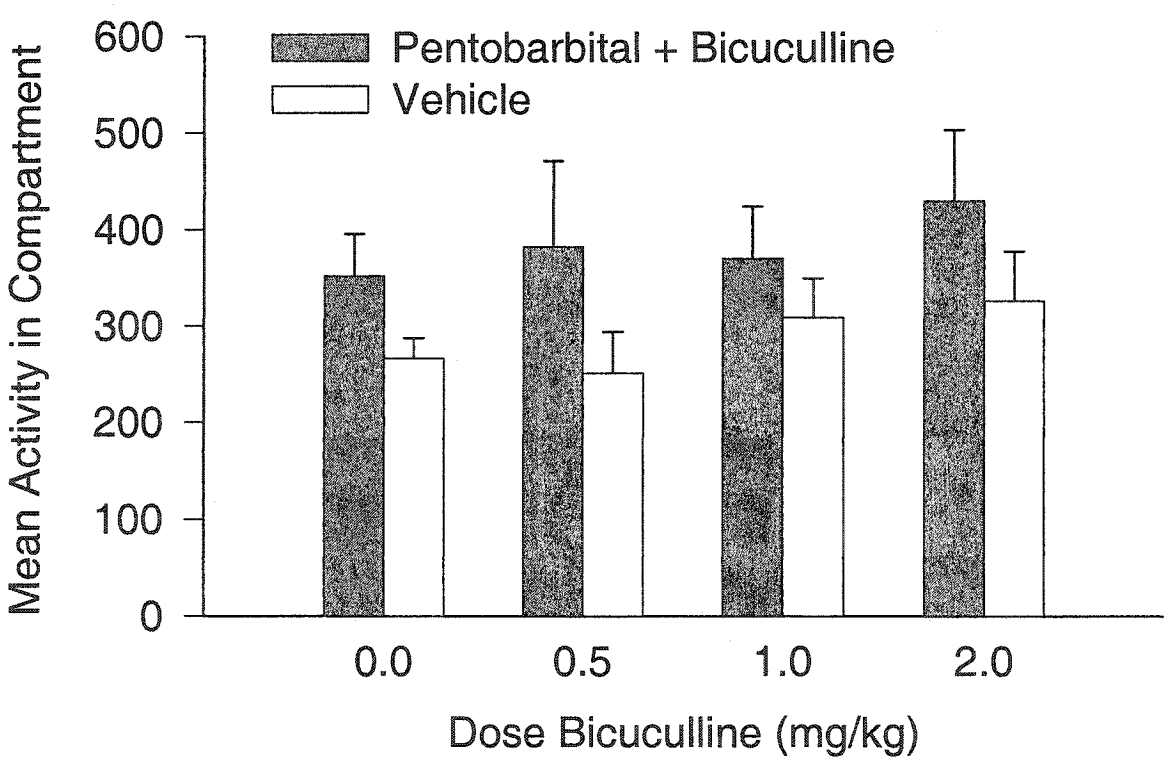
3.2. RESULTS

$GABA_A$ Receptor Antagonism of the Pentobarbital CPP

Rats spent significantly more time in the compartment paired with pentobarbital, or with pentobarbital plus 0.5 mg/kg bicuculline, than in the corresponding vehicle-paired compartment ($F_{(1,28)} = 7.00$, $p < 0.025$, $F_{(1,28)} = 9.02$, $p < 0.01$, respectively). Bicuculline pretreatment at 1.0 and 2.0 mg/kg eliminated the pentobarbital place preference, indicated by no significant difference in the time spent in the drug-paired compartment compared to the vehicle-paired compartment at these doses (Figure 3, upper panel). There were no significant locomotor activity effects of bicuculline pretreatment on the pentobarbital CPP

Figure 3. Upper panel: Mean time spent (seconds) in compartment paired with 0.0, 0.5, 1.0, and 2.0 mg/kg bicuculline pretreatment to 15 mg/kg pentobarbital (black bars) or vehicle (white bars). Lower panel: Mean activity counts in compartment paired with 0.0, 0.5, 1.0, and 2.0 mg/kg bicuculline pretreatment to 15 mg/kg pentobarbital (gray bars) or vehicle). *Vertical lines* mark the standard error of means (SEM). *Asterisks* indicate a significant difference ($p < 0.05$) between drug and saline condition.


Pentobarbital + Bicucculine
Vehicle
Mean Seconds in Compartment
600
500
400
300
200
100
0
*
*
0.0
0.5
1.0
2.0


Pentobarbital + Bicuculline
Vehicle
Mean Activity in Compartment
600
500
400
300
200
100
0
0.0
0.5
1.0
2.0
Dose Bicuculline (mg/kg)

(Figure 3, lower panel).

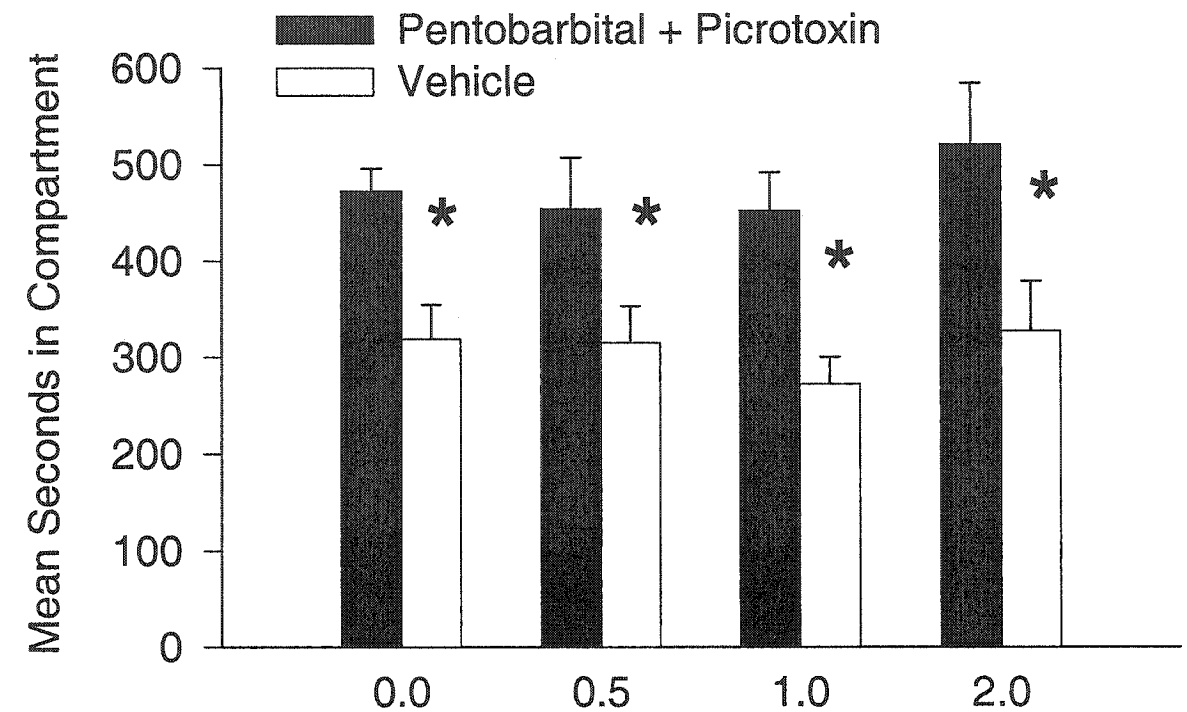
Picrotoxin did not interfere with the pentobarbital-induced CPP. The rats spent significantly more time in the compartments paired with pentobarbital alone ($F_{(1,28)} = 4.57$, $p < 0.05$), or in the compartments paired with pentobarbital plus each dose of picrotoxin: 0.5 mg/kg ($F_{(1,28)} = 6.47$, $p < 0.025$), 1.0 mg/kg ($F_{(1,28)} = 6.31$, $p < 0.025$), and 2.0 mg/kg ($F_{(1,28)} = 7.28$, $p < 0.025$), than in the vehicle-paired compartments (Figure 4, upper panel). With regards to locomotor activity, planned comparisons revealed that the rats were significantly more active on test day in the compartments paired with pentobarbital plus control and all doses of picrotoxin: 0 mg/kg ($F_{(1,28)} = 4.848$, $p < 0.05$), 0.5 mg/kg ($F_{(1,28)} = 3.889$, $p < 0.05$), 1.0 mg/kg ($F_{(1,28)} = 2.854$, $p < 0.05$), and 2.0 mg/kg picrotoxin ($F_{(1,28)} = 5.542$, $p < 0.05$) (Figure 4, lower panel).

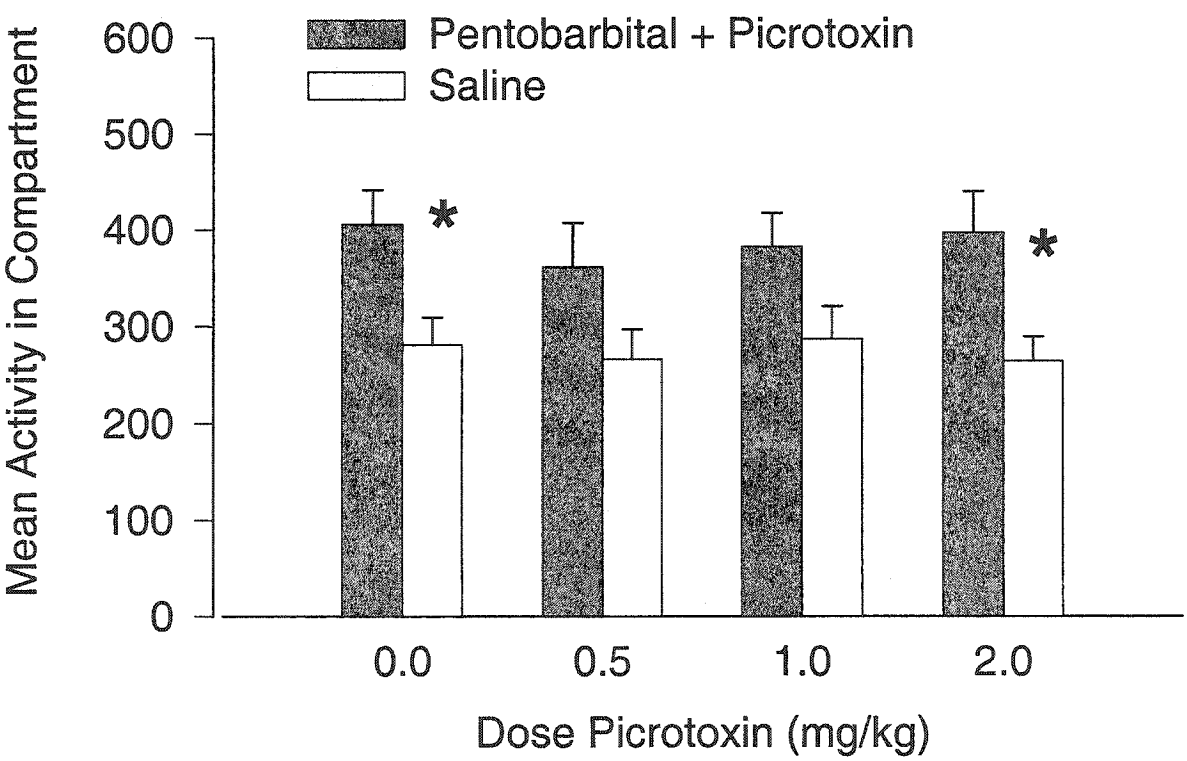
Neither 2.0 mg/kg bicuculline ($t = -1.125$, $p = 0.298$) nor 2.0 mg/kg picrotoxin ($t = -0.443$, $p = 0.67$) produced a place preference or aversion when administered alone (Figure 5).

Dopamine Antagonism of the Amphetamine and Pentobarbital CPP

Planned comparisons revealed that the amphetamine CPP ($F_{(1,28)} = 15.019$, $p < 0.01$) was blocked by the highest dose of eticlopride pretreatment (0.25 mg/kg) (Figure 6, upper panel). The preference was not blocked by 0.01 or 0.05 mg/kg eticlopride, since the rats spent significantly more time in the drug-paired compartment compared to the vehicle-paired compartment ($F_{(1,28)} = 16.466$, $p < 0.01$ and $F_{(1,28)} = 5.254$, $p < 0.05$, respectively). For locomotor activity, planned comparisons revealed that the rats were

Figure 4. Upper panel: Mean time spent (seconds) in compartment paired with 0.0, 0.5, 1.0, and 2.0 mg/kg picrotoxin pretreatment to 15 mg/kg pentobarbital (black bars) or vehicle (white bars). Lower panel: Mean activity counts in compartment paired with 0.0, 0.5, 1.0, and 2.0 mg/kg picrotoxin pretreatment to 15 mg/kg pentobarbital (gray bars) or vehicle (white bars). *Vertical lines* mark the standard error of means (SEM). *Asterisks* indicate a significant difference ($p < 0.05$) between drug and saline condition.


Pentobarbital + Picrotoxin
Vehicle
Mean Seconds in Compartment
600
500
400
300
200
100
0
*
*
*
*
0.0
0.5
1.0
2.0


Pentobarbital + Picrotoxin
Saline
Mean Activity in Compartment
600
500
400
300
200
100
0
*
*
0.0
0.5
1.0
2.0
Dose Picrotoxin (mg/kg)

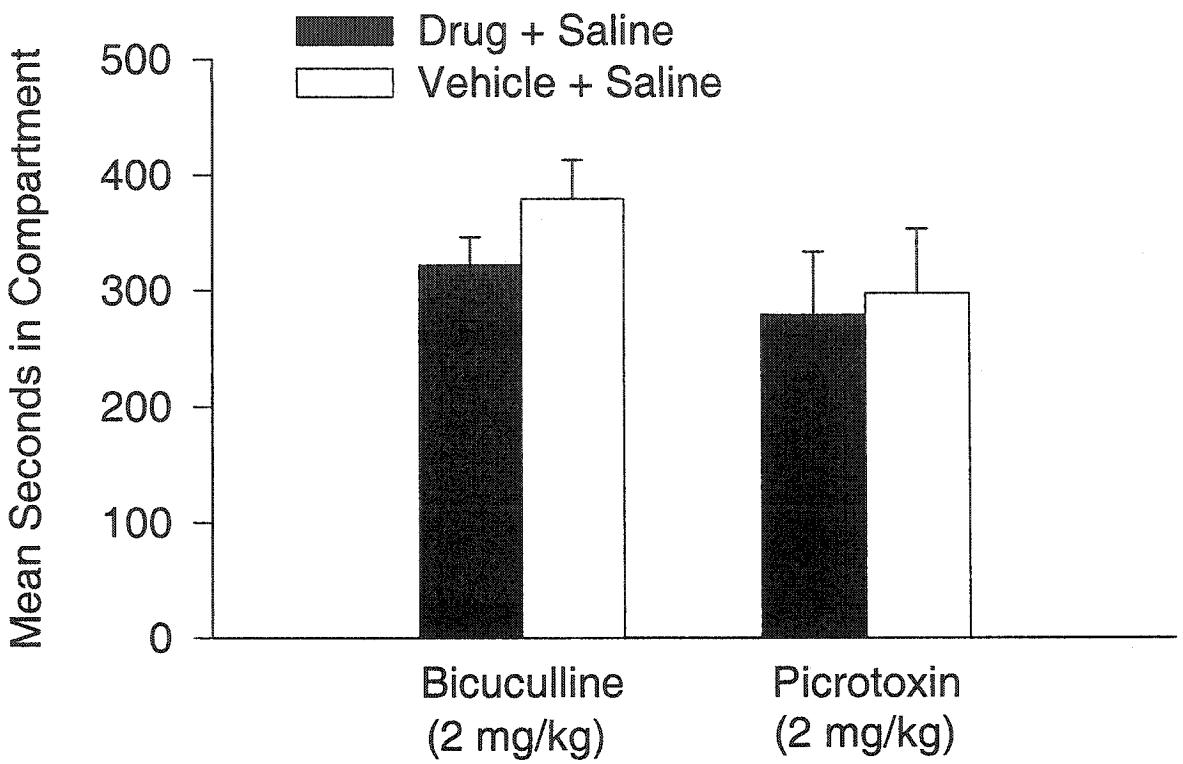
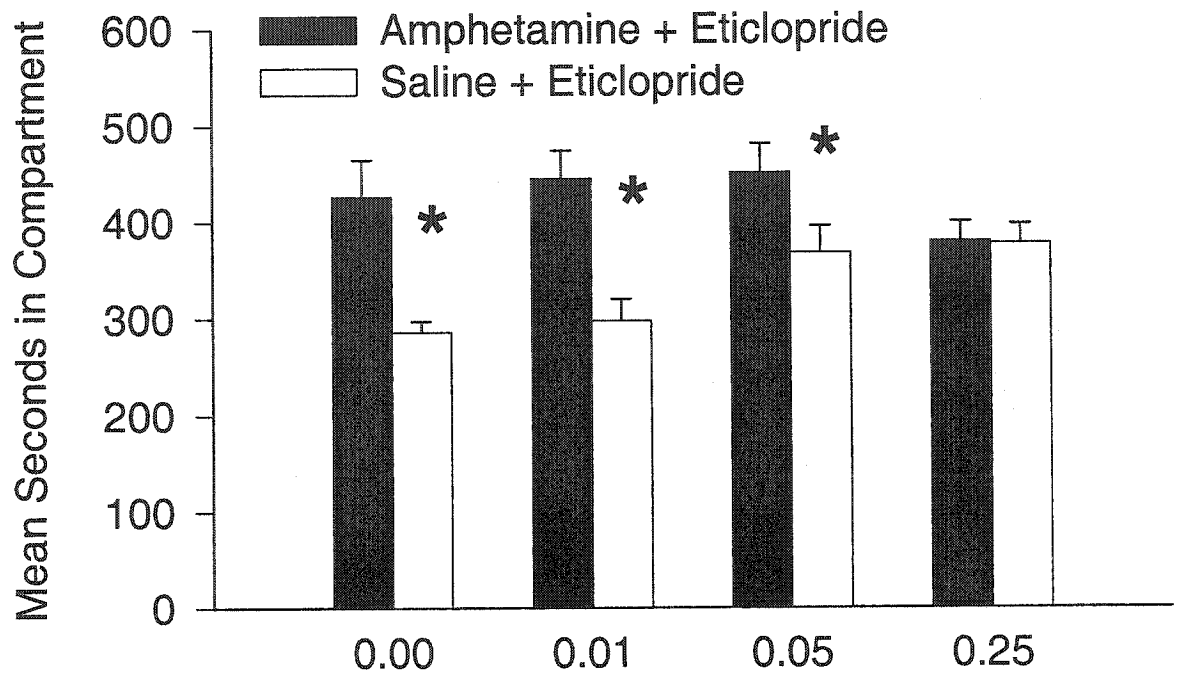


Figure 5. Mean time spent (seconds) in compartments paired with bicuculline (2.0 mg/kg) pretreatment or picrotoxin (2.0 mg/kg) pretreatment to saline (black bars) or vehicle pretreatment to saline (white bars). *Vertical lines* mark the standard error of the means (SEM). *Asterisks* indicate a significant difference ($p < 0.05$) between drug and vehicle condition.

Figure 6. Upper panel: Mean time spent (seconds) in compartment paired with 0.0, 0.01, 0.05, and 0.25 mg/kg eticlopride pretreatment to 1 mg/kg amphetamine (black bars) or eticlopride pretreatment to saline (white bars). Lower panel: Mean activity counts in compartment paired with 0.0, 0.01, 0.05, and 0.25 mg/kg eticlopride pretreatment to 1 mg/kg amphetamine (gray bars) or eticlopride pretreatment to saline (white bars). *Vertical lines* mark the standard error of means (SEM). *Asterisks* indicate a significant difference ($p < 0.05$) between drug and saline condition.


Amphetamine + Eticlopride
Saline + Eticlopride
Mean Seconds in Compartment
600
500
400
300
200
100
0
*
*
*
0.00
0.01
0.05
0.25

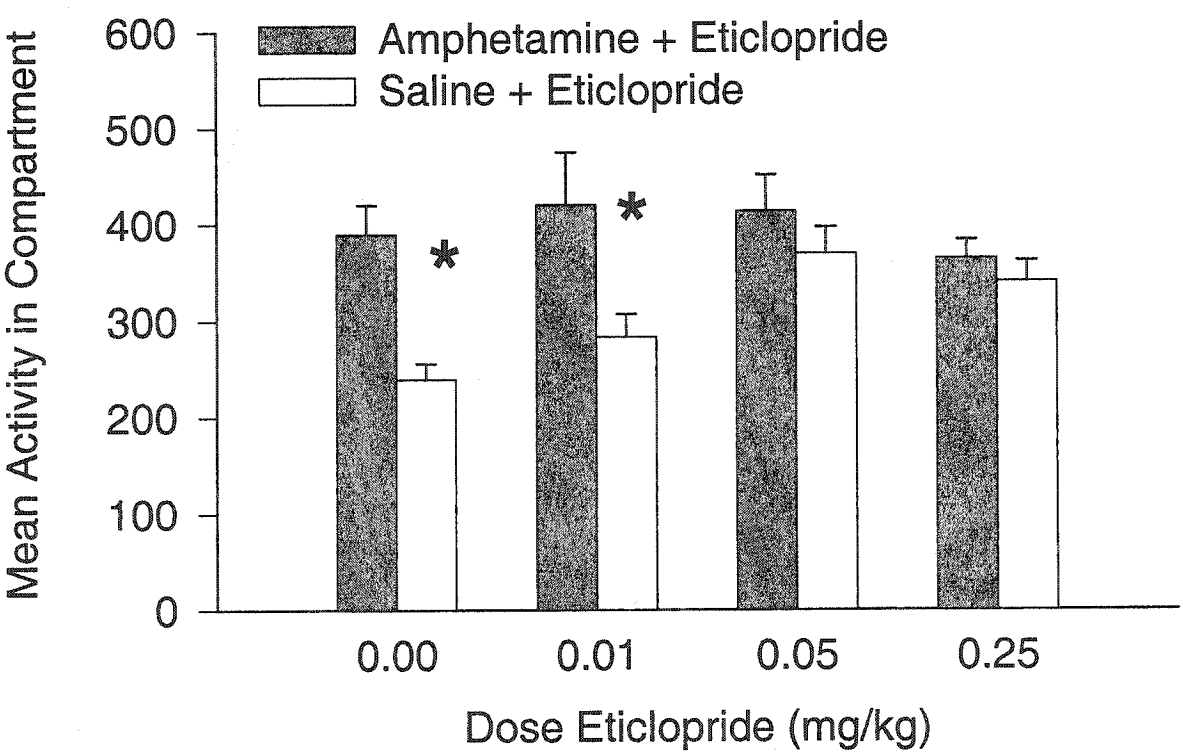
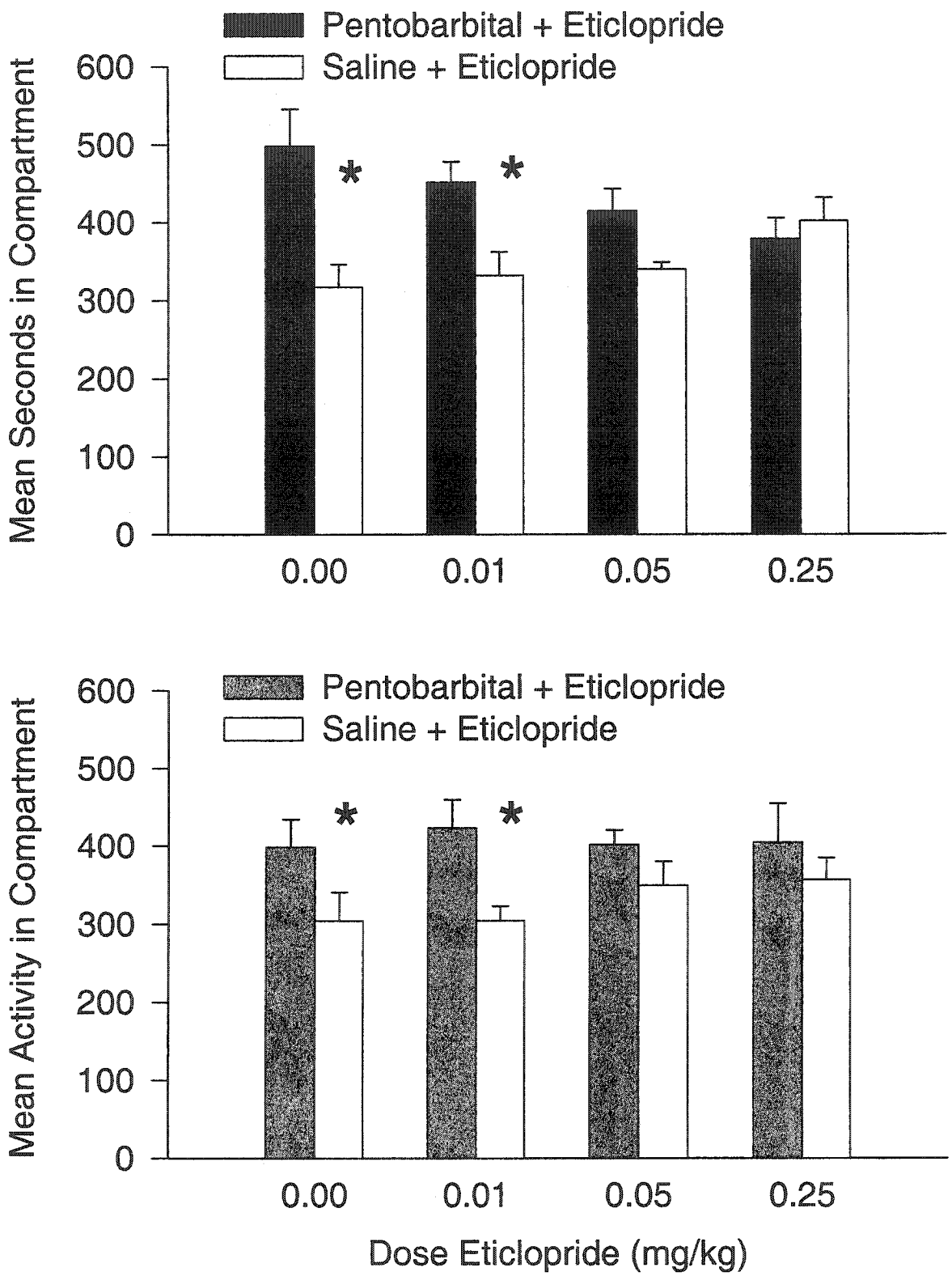

Amphetamine + Eticlopride
Saline + Eticlopride
Mean Activity in Compartment
600
500
400
300
200
100
0
*
*
0.00
0.01
0.05
0.25
Dose Eticlopride (mg/kg)

Figure 7. Upper panel: Mean time spent (seconds) in compartment paired 0.0, 0.01, 0.05, and 0.25 mg/kg eticlopride pretreatment to 15 mg/kg pentobarbital (black bars) or eticlopride pretreatment to saline (white bars). Lower panel: Mean activity counts in compartment paired with 0.0, 0.01, 0.05, and 0.25 mg/kg eticlopride pretreatment to 15 mg/kg pentobarbital (gray bars) or eticlopride pretreatment to saline (white bars). *Vertical lines* mark the standard error of means (SEM). *Asterisks* indicate a significant difference ($p < 0.05$) between drug and saline condition.


Pentobarbital + Eticlopride
Saline + Eticlopride
Mean Seconds in Compartment
600
500
400
300
200
100
0
*
*
0.00
0.01
0.05
0.25
Pentobarbital + Eticlopride
Saline + Eticlopride
Mean Activity in Compartment
600
500
400
300
200
100
0
*
*
0.00
0.01
0.05
0.25
Dose Eticlopride (mg/kg)

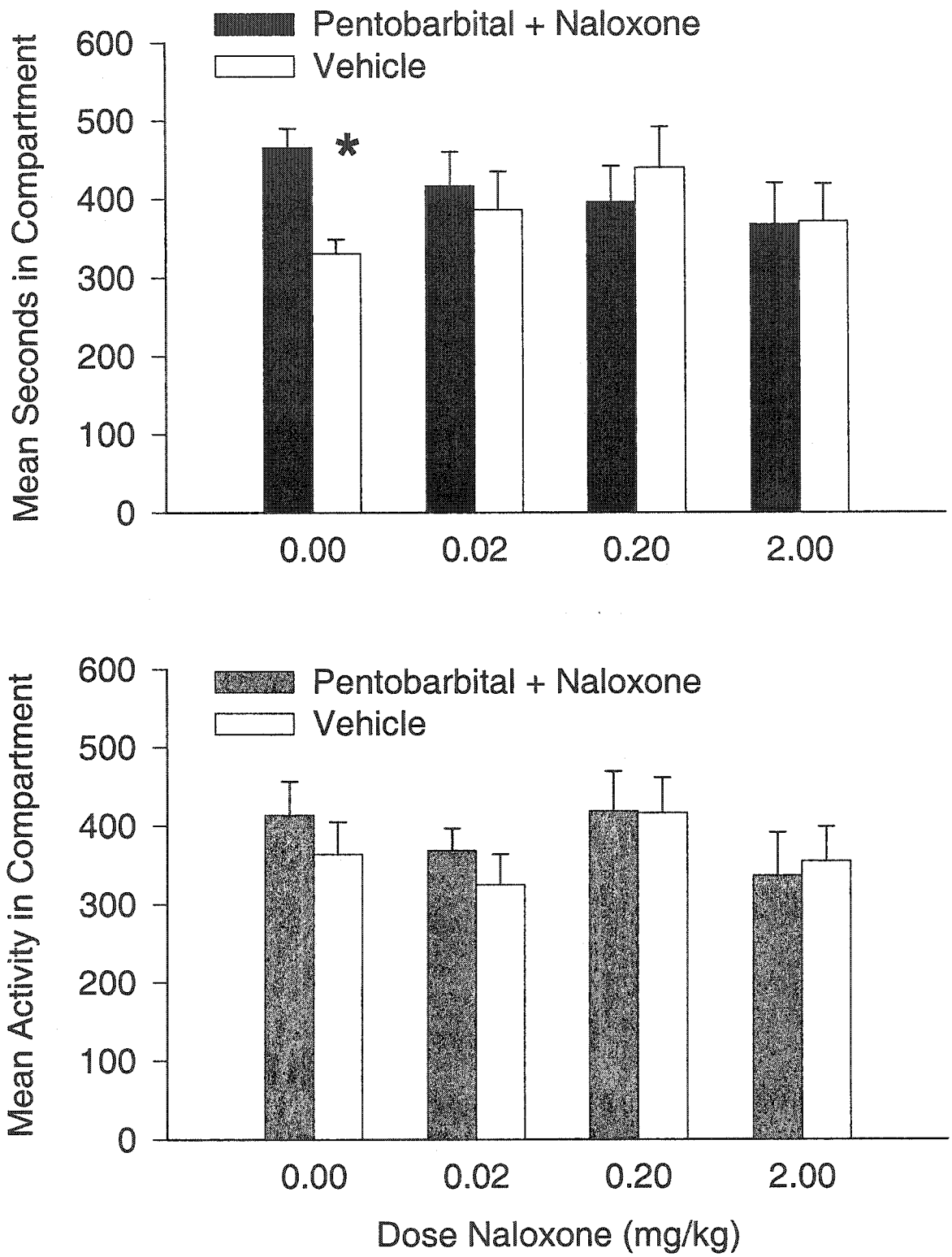
significantly more active on test day in the compartments paired with amphetamine plus 0 mg/kg ($F_{(1,28)} = 9.761$, $p < 0.01$) and 0.01 mg/kg eticlopride ($F_{(1,28)} = 8.145$, $p < 0.01$; Figure 6, lower panel).

For eticlopride pretreatment to the 15 mg/kg pentobarbital-induced CPP, the data for time spent in the drug- and vehicle-paired compartments were logarithmically transformed because the error variances were unequal (Levene's test: $F = 3.16$, $p < 0.05$). The pentobarbital CPP ($F_{(1,28)} = 11.10$, $p < 0.01$) was blocked when pentobarbital was combined with eticlopride pretreatment at 0.05 and 0.25 mg/kg (i.e. no significant difference for time spent in the drug-paired compartment compared to the vehicle-paired compartment) (Figure 7, upper panel). The preference was not blocked by pretreatment with 0.01 mg/kg eticlopride, since at this dose of antagonist, the rats spent significantly more time in the drug-paired compartment than in the vehicle-paired compartment ($F_{(1,28)} = 5.87$, $p < 0.025$). With regards to locomotor activity, planned comparisons revealed that activity was blocked by the same eticlopride doses (0.05 and 0.25 mg/kg) that attenuated time spent in the compartments, but activity was not blocked with pentobarbital plus 0 mg/kg ($F_{(1,28)} = 3.609$, $p < 0.05$) or 0.01 mg/kg eticlopride ($F_{(1,28)} = 6.117$, $p < 0.025$) (Figure 7, lower panel).

Opioid Antagonism of the Pentobarbital CPP

The pentobarbital place preference ($F_{(1,28)} = 4.51$, $p < 0.05$) was blocked by naloxone at all doses tested (0.02, 0.2, and 2.0 mg/kg), indicated by no significant difference in the time spent in the drug-paired compartment compared to the vehicle-

Figure 8. Upper panel: Mean time spent (seconds) in compartment paired with 0.0, 0.02, 0.2, and 2.0 mg/kg naloxone pretreatment to 15 mg/kg pentobarbital (black bars) or vehicle (white bars). Lower panel: Mean activity counts in compartment paired with 0.0, 0.02, 0.2, and 2.0 mg/kg naloxone pretreatment to 15 mg/kg pentobarbital (gray bars) or vehicle (white bars). *Vertical lines* mark the standard error of means (SEM). *Asterisks* indicate a significant difference ($p < 0.05$) between drug and saline condition.


Pentobarbital + Naloxone
Vehicle
Mean Seconds in Compartment
600
500
400
300
200
100
0
*
0.00
0.02
0.20
2.00
Pentobarbital + Naloxone
Vehicle
Mean Activity in Compartment
600
500
400
300
200
100
0
0.00
0.02
0.20
2.00
Dose Naloxone (mg/kg)

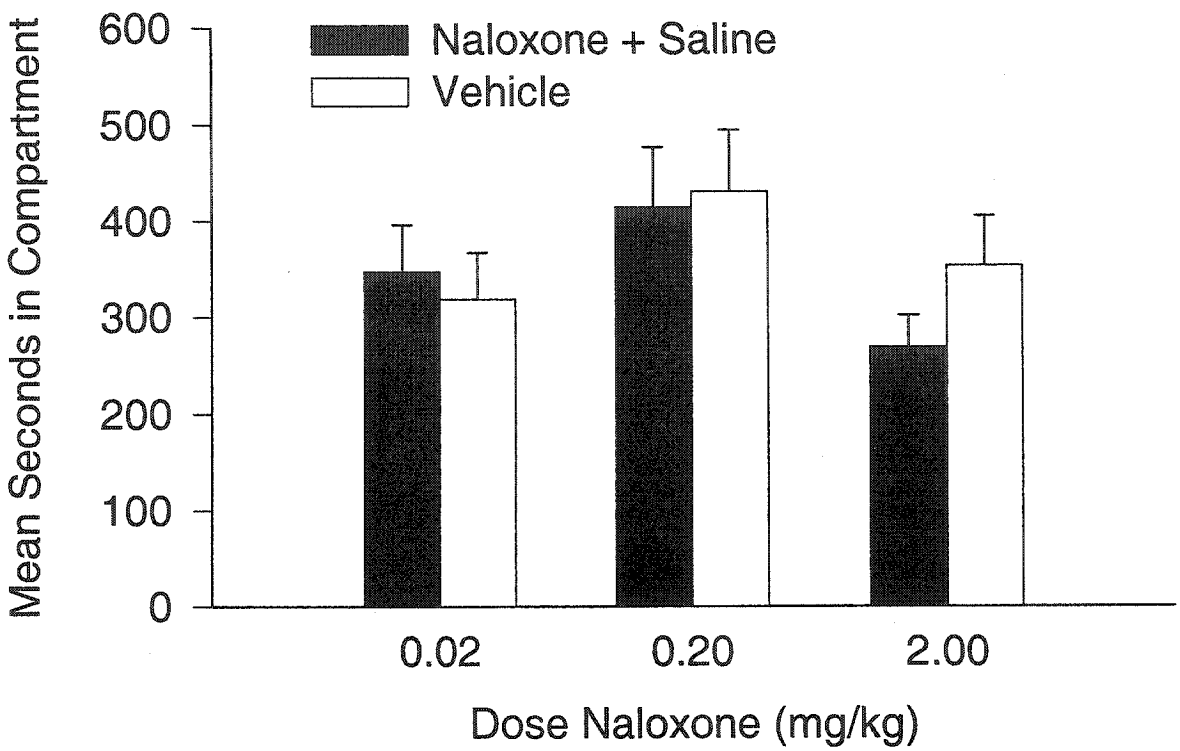


Figure 9. Mean time spent (seconds) in compartment paired with 0, 0.02, 0.2, and 2.0 mg/kg naloxone pretreatment to saline (black bars) or vehicle pretreatment to saline (white bars) . *Vertical lines* mark the standard error of the means (SEM). *Asterisks* indicate a significant difference ($p < 0.05$) between drug and vehicle condition.

paired compartment (Figure 8, upper panel). No significant differences were found for any of the groups with regards to locomotor activity (Figure 8, lower panel).

Naloxone administered by itself did not produce a significant place preference or place aversion at any of the doses tested: 0.02 mg/kg ($F_{(1,21)} = 0.104$, NS), 0.2 mg/kg ($F_{(1,21)} = 0.033$, NS), and 2.0 mg/kg ($F_{(1,21)} = 0.904$, NS) (Figure 9).

3.3. DISCUSSION

The place preference induced by 15 mg/kg pentobarbital was blocked by pretreatment of bicuculline (1.0 & 2.0 mg/kg), eticlopride (0.05 & 0.25 mg/kg), and naloxone (0.02, 0.2, & 2.0 mg/kg). Although the tests are not used here (see Section 2.1), there were in fact no significant differences between doses in these experiments. Such a pattern of results is not surprising given the greater power of repeated measure designs, and the small size of the pentobarbital CPP. Combining data across experiments for tests with pentobarbital alone (n = 40), the size of the pentobarbital CPP can be estimated as a preference of 158 seconds with a standard deviation of 139. To detect a difference (α = 0.05) of this magnitude 80% of the time requires samples of 9 subjects with a repeated measure, but 14 subjects with independent groups. Confirmation of the statistical analysis used here comes from replications. The CPP to 15 mg/kg pentobarbital was reliable, and was replicated 4 times in the antagonist pretreatment experiments. Furthermore, the soundness of conclusions drawn from the statistical analysis is reinforced by the consistency of the receptor antagonism results. In every case, the relationship of

significant differences to drug dose was pharmacologically meaningful in that low doses were ineffective and high doses were effective. Moreover, the locomotor activity results are consistent with the place findings in that doses that blocked the place preference also attenuated the locomotor activity, and the doses that did not block the place preference did not attenuate locomotor activity.

Bicuculline, a $GABA_A$ receptor antagonist, attenuated the pentobarbital place preference at 1.0 and 2.0 mg/kg but not at 0.5 mg/kg. Picrotoxin, also a $GABA_A$ receptor antagonist, did not block a place preference to 15 mg/kg pentobarbital at any of the doses tested (0.5, 1.0, and 2.0 mg/kg). Neither of these antagonists produced effects in the CPP task when administered on their own. The lack of effect of picrotoxin is not likely due to an inadequate dose because similar doses of picrotoxin are reported to block a CPP to diazepam (Spyraki et al., 1985) and antagonize the hypnotic effect of pentobarbital (Malcangio et al., 1992). Higher doses of picrotoxin could not be safely tested, since pilot studies indicated that 4 mg/kg induced convulsions in some rats.

While the finding that bicuculline, but not picrotoxin, blocked the pentobarbital CPP may seem surprising, it is consistent with other reports on the dissociable actions of these antagonists on barbiturate effects. Despite the fact that barbiturates and picrotoxin respectively stimulate and block the $GABA_A$ receptor (Nestoros, 1984), picrotoxin does not block all bicuculline-antagonizable effects of barbiturates. Thus, picrotoxin does not reverse the anticonvulsant effect of pentobarbital in the electroshock seizure model, while bicuculline does (Mehta and Ticku, 1986). Similarly, bicuculline, but not picrotoxin, antagonizes pentobarbital-induced depression of excitability in rat dentate gyrus granule

cells (Joy and Albertson, 1991).

In terms of other place preference effects, bicuculline injected into the VTA is reported to be ineffective in suppressing a morphine-induced CPP (Tsuji et al., 1996). In contrast to the current results, Chester and Cunningham (1999) found that when systemically administered, 1 mg/kg, but not 3 or 5 mg/kg bicuculline, increased the magnitude of ethanol-induced place preferences in mice (Chester and Cunningham, 1999). The authors suggest that the increased magnitude of the ethanol-induced CPP may be because $GABA_A$ receptors tonically inhibit the neural substrates that mediate the rewarding effects of ethanol in the CPP procedure. In the experiment by Chester and Cunningham, bicuculline was administered immediately before ethanol, and the conditioning trials were only 5 minutes. In the present experiment, bicuculline was administered 15 minutes prior to pentobarbital injections, since the antinociceptive effects of bicuculline in the hot plate test and its antagonist effects on pentobarbital-induced hypnosis are reported to be maximum at this time (Malcangio et al., 1992). Furthermore, the conditioning trials in the present experiment were 30 minutes. Given the time frame of the bicuculline injections and the short conditioning trials in the study by Chester and Cunningham (1999), it is possible that the peak effect of bicuculline occurred after the animals were removed from the conditioning compartments. Therefore, it is possible that blocking effects of bicuculline on the ethanol CPP were masked by the timing of pretreatment with bicuculline and by the short conditioning trials.

Eticlopride, a DA D_2 receptor antagonist, blocked the place preference to 1 mg/kg amphetamine and 15 mg/kg pentobarbital. The effect is unlikely to be due to an aversive

effect of eticlopride, since eticlopride does not produce a place preference or place aversion when tested on its own (Hoffman, 1994). These results suggest that DA plays a role in the reinforcing effects of pentobarbital in the CPP paradigm, as it does with other reinforcing drugs (Wise, 1989; Hiroi and White, 1990). It also suggests the possibility that barbiturates, like other drugs of abuse, are able to enhance DA activity in the NAc (Wise, 1987). Although barbiturates are classified as sedatives, low doses of barbiturates are reported to stimulate locomotor activity in mice and operant behaviour in rats (Wenger, 1986), and this effect is consistent with them having weak psychostimulant properties at some doses. However, the effects of barbiturates on the catecholaminergic system are at best unclear. Barbiturates decrease the turnover of cortical noradrenergic nerve terminals and neostriatal DA nerve terminals in unstressed rats (Lidbrink et al., 1972), and acute administration of pentobarbital significantly elevates DA concentrations in the cortex and striatum (Nabeshima et al., 1981). *In vivo* dialysis in the NAc suggests that lower doses (0.75 mg/kg) enhance DA release from the NAc, but higher doses of pentobarbital (5 mg/kg) depress DA release (Di Chiara and Imperato, 1986). Moreover, Di Chiara and Imperato report that the dose that stimulated DA release also produced behavioural stimulation, while the dose that depressed DA release produced sedation and hypnosis. The finding that 15 mg/kg pentobarbital produced a significant CPP is not consistent with the biochemical results, since at this dose, pentobarbital should depress DA release from the NAc. However, the locomotor activity data on test day indicates that this dose produced an increase in activity when the rats were exposed to the conditioned stimulus. Furthermore, the 15 mg/kg pentobarbital CPP is consistent with behavioural

findings with other drugs of abuse in the place preference paradigm. Researchers report that stronger place preferences are obtained with sedative doses of opiates than with locomotor-stimulating doses (van der Kooy, 1987; Bozarth, 1987b), and with stereotypy-inducing doses of psychostimulants than with locomotor-inducing doses (Bardo et al., 1999).

The effects of naloxone were also consistent with the hypothesis that pentobarbital reinforcement is mediated by the neural mechanisms common to other drugs of abuse. In this experiment, naloxone blocked the pentobarbital CPP as it does opiate-mediated place preferences, such as morphine (Phillips and LePiane, 1980), and dopamine-mediated place preferences, such as cocaine (Gerrits et al., 1995; Kim et al., 1997), amphetamine (Trujillo et al., 1991), and fencamfamine (Planeta et al., 1995). Few studies have examined the dose-response relationship for naloxone antagonism of drug-induced CPPs. However, the lowest effective dose (0.02 mg/kg) that blocked the pentobarbital-induced CPP is comparable to the lowest effective dose reported to block amphetamine (Trujillo et al., 1991). Naloxone administered by itself did not produce a significant place aversion at any of the doses tested, indicating that the antagonism of the pentobarbital-induced CPP is not due to summation of rewarding and aversive drug effects. In previous studies, conflicting results have been reported, with some studies observing place aversions (Mucha and Iversen, 1984) and other studies reporting no effect (Phillips and LePiane, 1980; Bozarth and Wise, 1981).

In conclusion, the reinforcing effects of pentobarbital in the place preference test appear to depend on GABAergic, dopaminergic, and opioid systems. While it is not

likely, these reinforcing effects could be peripherally mediated. Alternatively, peripheral effects could be aversive and reduce the size of the CPP (Bechara and van der Kooy, 1985). To examine this issue, the next chapter tests whether a barbiturate that is more suitable for intracranial injections than pentobarbital will induce a CPP when centrally administered.

CHAPTER 4:

SODIUM BARBITAL-INDUCED PLACE PREFERENCE: EFFECTS OF SYSTEMIC AND INTRACEREBROVENTRICULAR ADMINISTRATION

It is possible that the investigation of the neuropharmacology of barbiturate reinforcement has been hindered by the fact that most barbiturates are not ideal agents for direct intracranial administration (Lyness et al., 1979a). Many barbiturates, such as pentobarbital, are relatively insoluble in water at a neutral pH, and aqueous solutions are very alkaline (Budavari and O'Neil, 1996). However, the long-acting barbiturate, sodium barbital, is soluble at a neutral pH and has been used as a buffer in the 7.0 - 9.0 range of pH (Lillie, 1965).

Pharmacologically, barbital is similar to other barbiturates in that its primary action is through the GABAergic system. Barbital potentiates GABA-activated currents in rod bipolar cells of the rabbit retina (Gillette and Dacheux, 1995) and enhances muscimol-induced stimulation of $^{36}Cl^-$ flux in brain homogenates of both alcohol-sensitive and alcohol-insensitive rats (Uusi-Oukari and Korpi, 1992). Additionally, like pentobarbital, chronic exposure to barbital decreases the enhancement of [^{3}H]flunitrazepam binding, which is consistent with an allosteric uncoupling of GABA and benzodiazepine recognition sites of the $GABA_A$ receptor (Roca et al., 1990).

When chronically administered, barbital produces functional tolerance and physical dependence in animals (Wahlstrom, 1979; Okamoto and Hinman, 1984). In terms of reinforcing effects, barbital is self-administered by rhesus monkeys (Winger et al., 1975) and rats under certain environmental conditions (Zimmerberg and Brett, 1992), and is reported to be abused by humans (Bailey and Jatlow, 1975). Furthermore, rats discriminate 80 mg/kg barbital from control (saline) injections (York, 1978) and 56 mg/kg barbital produces pentobarbital-like discriminative responding in both rhesus monkeys

(Winger and Herling, 1982) and pigeons (Herling et al., 1980).

Given that barbital shares a common mode of pharmacological action to pentobarbital and, like pentobarbital, is self-administered by animals and humans, it was proposed that systemic administration of barbital would also produce a place preference. Since barbital is a barbiturate suitable for intracranial injection, intracerebroventricular (ICV) administration of barbital was also examined in the CPP task.

4.1. METHODS

Statistical Analysis

See Section 2.1.

4.1.1. Systemic Dose-Response Place Preference

Animals

See Section 2.1.

Apparatus

The CPP apparatus was identical to the one used in the Sections 2.1 and 3.1., with one exception. In the experiments in this and subsequent chapters, compartments A and B differed in floor texture and brightness in addition to shading differences. Thus, the floor and ceiling of compartment A was painted black with black and white horizontal stripes on

the walls, it had a smooth floor, and a clear plexiglass front wall. The floor and ceiling of compartment B were painted white with black and white vertical stripes on the walls, it had a wire mesh floor, and a darkly tinted plexiglass front wall.

Place Conditioning Procedure

Behavioural testing and conditioning was identical to the procedure described in Section 2.1 except for that training days were separated by 48 hours, instead of 24 hours. The increased time interval between each manipulation for the systemic barbital experiments was to allow for drug clearance between conditioning trials, since barbital has an elimination half-life of 13 to 20 hours in the rat (Flynn and Spector, 1972;Khanna et al., 1980). Pre-exposure, conditioning, and test sessions were the same as in the systemic pentobarbital experiments.

Sixty rats were randomly assigned to a control group (0 mg/kg) or one of four dose groups of barbital: 2.7, 8, 24, or 72 mg/kg (n = 12 for each dose). Sodium barbital (Veronal®, Sigma) was dissolved with dH_20 and 0.3M HCl was added to reach a pH of 7.8 - 8.0. It was prepared fresh daily. Barbital was injected IP (3 ml/kg) and dH_20 (pH ≈ 8.0) was injected IP during drug free conditioning trials.

4.1.2. Intracerebroventricular Conditioned Place Preference

Animals

Adult male Long Evans rats individually housed and weighing between 250 and

275 grams at the beginning of the experiment were used.

Surgery

All surgical procedures were conducted under xylazine (AnaSed™, 2 mg/kg IM) and sodium pentobarbital (Somnital®, 45 mg/kg IP) anesthesia. Atropine sulphate (0.1 mg/kg SC) and Trimethoprim sulfadiazine (24% Tribrissin®, 2 mg/kg SC) were administered pre-operatively and trimethoprim sulfadiazine was also administered the day following surgery. Permanent indwelling stainless steel (23-gauge) guide cannula were implanted bilaterally 1.5 mm above the lateral ventricles, according to the atlas of (Paxinos and Watson, 1998). Stereotaxic coordinates from Bregma were AP −1.0, ML ±1.4, DV −3.0 mm (flat skull). Cannula were secured to the skull with stainless steel screws and dental acrylic. Stainless steel stylets (00-gauge insect pins), 1 mm longer than the guide cannula, were inserted into the guide cannula to keep them free of debris. Animals were administered dipyrone (100 mg/kg SC) approximately 2 hours following surgery and were allowed to recover 7 - 10 days before behavioural testing began.

Apparatus and Place Conditioning Procedure

The apparatus and conditioning procedure was the same as for the systemic dose-response CPP (Section 4.1.1.) with the exception that 24 hours elapsed between each training manipulation.

Intracerebroventricular (ICV) injections were made through 30-gauge stainless-steel injectors that fitted into the guide cannulas and extended 1.5 mm beyond the cannula

tips. Injectors were connected to a length of polyethylene tubing (PE-20) filled with drug or vehicle by means of a dual syringe pump (Harvard Apparatus, South Natick, MA). The progress of drug or vehicle infusion was monitored by the movement of small air bubbles in the tubing.

Barbital was dissolved as in the preceding experiment. Rats were randomly assigned to a control group (0 μg) or one of four dose groups of barbital: 60, 120, 240, or 480 μg. 60, 120, and 240 μg were dissolved in12 μl and were injected bilaterally at a rate of 3 μl/minute for 2 minutes (6 μl per side). To overcome saturation limit, 480 μg was dissolved in 20 μl and was injected at a rate of 5 μl/minute for 2 minutes (10 μl per side). Inner cannula were left in place for an additional minute to reduce the possibility of reflux up the cannula.

Histology

After the completion of behavioural testing, 1 μl of Indian ink (Speedball India Ink, Statesville, NC, USA) was microinjected through the guide cannula. Subjects were deeply anesthetized with 30 % chloral hydrate and perfused through the heart with 0.9% saline followed by 10% formalin. Brains were removed and stored in 10% formalin for at least 24 hours. Brains were sliced in the coronal plane (30 μm sections) and stained with Cresyl Violet (Cellpoint Scientific Inc., Rockville, MD). Data from individual subjects were discarded if the injections were unilateral or fell outside of the ventricles.

4.2. RESULTS

Systemic Dose-Response Barbital CPP

Planned comparisons revealed that the rats spent significantly more time in the compartment paired with 8 and 24 mg/kg barbital ($F_{(1,55)} = 4.43$, $p < 0.05$ and $F_{(1,55)} = 3.10$, $p < 0.05$, respectively), but not with 0, 2.7, or 72 mg/kg barbital ($F_{(1,55)} = 2.47$, NS; $F_{(1,55)} = 0.80$, NS; $F_{(1,55)} = 0.17$, NS, respectively; Figure 10, upper panel).

With regard to locomotor activity, no significant findings were obtained for the within-subject comparisons (Figure 10, lower panel).

ICV Barbital CPP

Cannula were unilateral in four rats and there was no histology for two other rats. The data from these rats were not included in the analyses, and the final number of animals was 10-12 per group. Planned comparisons revealed that the rats spent significantly more time in the compartment paired with 240 or 480 ($F_{(1,51)} = 4.68$, $p < 0.05$) and $F_{(1,51)} = 8.12$, $p < 0.01$, respectively), but not with 0, 60, or 120 μg barbital ($F_{(1.51)} = 2.18$, NS; $F_{(1,51)} = 0.18$, NS; $F_{(1,51)} = 0.16$, NS, respectively; Figure 11, upper panel).

In terms of locomotor activity, planned comparisons revealed that the rats were significantly more active only in the compartment paired with 480 μg barbital ($F_{(1,51)} = 4.412$, $p < 0.05$; Figure 11, lower panel).

Figure 10. Upper panel: Mean time spent (seconds) in compartment paired with 0, 2.7, 8, 24, and 72 mg/kg barbital (black bars) or vehicle (white bars). Lower panel: Mean activity counts in compartment paired with 0, 2.7, 8, 24, and 72 mg/kg barbital (gray bars) or vehicle (white bars). *Vertical lines* mark the standard error of means (SEM). *Asterisks* indicate a significant difference ($p < 0.05$) between drug and saline condition.

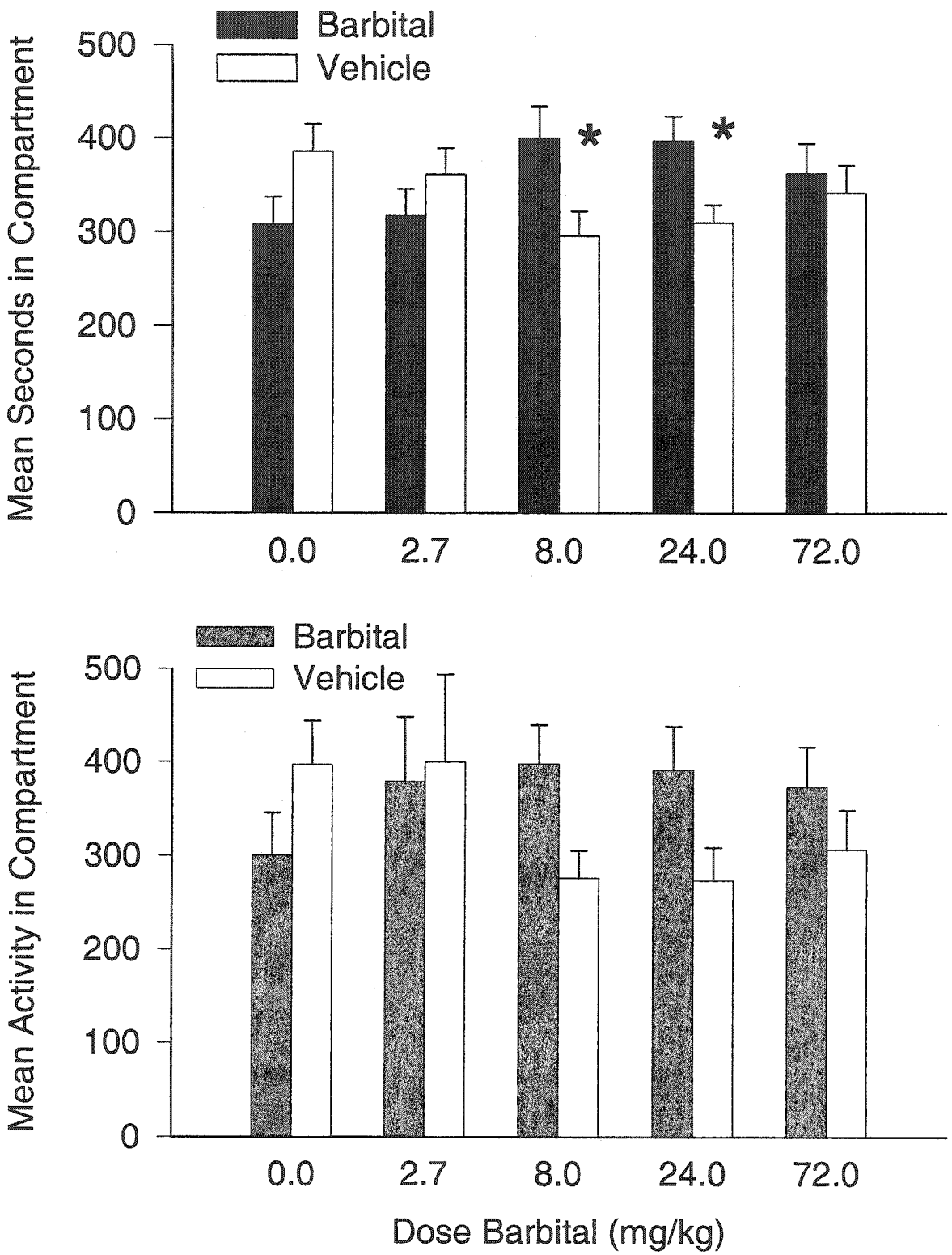
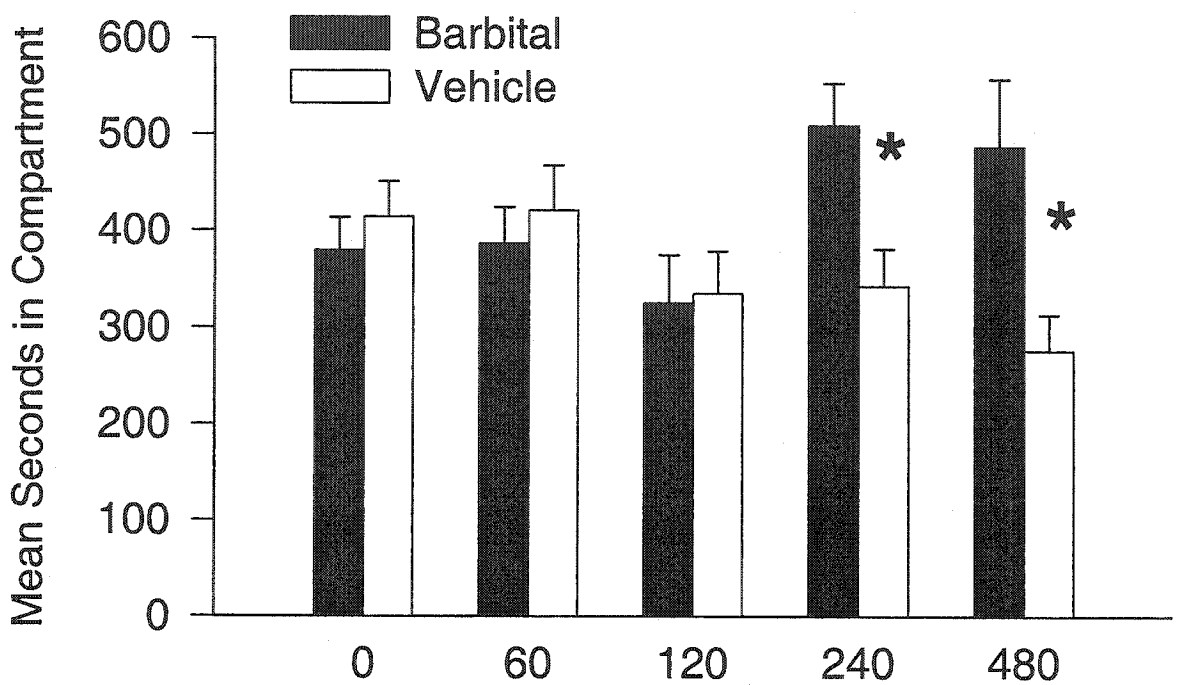
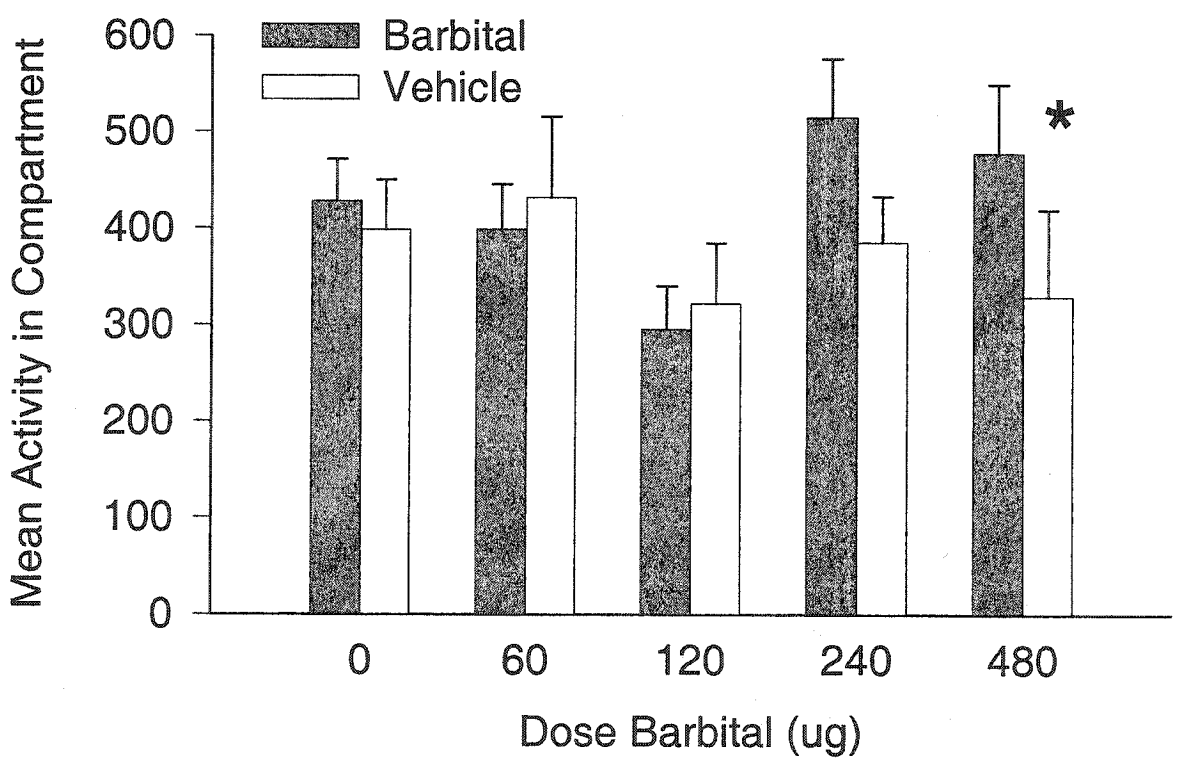

Barbital
Vehicle
Mean Seconds in Compartment
500
400
300
200
100
0
*
*
0.0
2.7
8.0
24.0
72.0
Barbital
Vehicle
Mean Activity in Compartment
500
400
300
200
100
0
0.0
2.7
8.0
24.0
72.0
Dose Barbital (mg/kg)

Figure 11. Upper panel: Mean time spent (seconds) in compartment paired with 0, 60, 120, 240, and 480 µg intracerebroventricular (ICV) barbital (black bars) or vehicle (white bars). Lower panel: Mean activity counts in compartment paired with 0, 60, 120, 240, and 480 µg ICV barbital (gray bars) or vehicle). *Vertical lines* mark the standard error of means (SEM). *Asterisks* indicate a significant difference ($p < 0.05$) between drug and saline condition.


Barbital
Vehicle
Mean Seconds in Compartment
600
500
400
300
200
100
0
*
*
0
60
120
240
480


Barbital
Vehicle
Mean Activity in Compartment
600
500
400
300
200
100
0
*
0
60
120
240
480
Dose Barbital (ug)

4.3. DISCUSSION

When systemically and centrally administered, barbital was reinforcing in the CPP paradigm and produced dose-dependent place preferences. This is consistent with findings that barbital is reinforcing in laboratory animals in the self-administration paradigm (Winger et al., 1975; Zimmerberg and Brett, 1992), and has abuse potential in humans (Bailey and Jatlow, 1975). Moreover, it demonstrates that a barbiturate other than pentobarbital is reinforcing in the CPP paradigm.

In the systemic barbital experiment, the rats spent significantly more time in the barbital-paired compartment compared to the vehicle-paired compartment only at 8 and 24 mg/kg, but not 2.7 or 72 mg/kg. This result is comparable to that obtained with the pentobarbital-induced place preference in that the CPP is obtained only within a narrow dose range (see Chapter 2). Moreover, similarities between pentobarbital and barbital were noted for the behavioural effects of the drugs (personal observations). The highest doses of these drugs produced sedation and motor ataxia in the rats, whereas the doses that produced significant place preferences produced moderate (pentobarbital), or slight (barbital), signs of motor impairment. This is also consistent with reports that opiates induce a stronger place preference with sedative, rather than locomotor-stimulating, doses (van der Kooy, 1987; Bozarth, 1987b). It should be noted, however, that locomotor activity was elevated in the compartment associated with the effective doses of barbital (see lower panels of Figure 10 and 11), which may be a response to the conditioned effects of the drug (e.g. drug-seeking behaviour).

According to the literature, pentobarbital is faster acting and approximately 10 times more potent than barbital. Boisse and Okamoto (1978) found that the time required to achieve anaesthesia was 30-60 minutes for barbital compared to 3-10 minutes for pentobarbital. In the present study, pentobarbital and barbital were approximately equipotent when administered systemically. This is somewhat surprising in view of reports that drug responding increased and was maintained by 0.25 - 4.0 mg/kg/infusion for pentobarbital and 2.5 - 10 mg/kg/infusion for barbital (Winger et al., 1975), which reflects a 10 fold difference between the reinforcing doses of the barbiturates. The reason for the discrepancy between the reinforcing dose range of pentobarbital and barbital in the two paradigms is not known, but may be related to drug absorption and route of administration (intravenous for self-administration vs. intraperitoneal for CPP). Furthermore, it should be noted that discrepancies for drug potency between these paradigms are also reported for opiate drugs. Whereas intravenous self-administration of heroin (0.05 - 0.2 mg/kg/infusion) (Wise et al., 1995) and morphine (0.2 - 0.4 mg/kg/infusion) (Pontieri et al., 1995) reflects a 4 fold difference, heroin and morphine are like barbiturates in that they are more equipotent in the CPP test (e.g. 0.25 - 2.0 mg/kg for heroin (Amalric et al., 1987; Bozarth, 1987a) and 0.2 - 5.0 mg/kg for morphine (Mucha and Iversen, 1984).

Finally, although it is assumed that the effects of the systemic administration of drugs on reinforcement tasks are produced centrally, it is possible that reinforcing effects are mediated by peripheral mechanisms. The present finding demonstrates that the reinforcing effects of barbital in the CPP task are centrally mediated. ICV barbital (240

and 480 μg) induced a significant CPP, and this finding is consistent with ICV-induced place preferences of other drugs of abuse, such as cocaine (Morency and Beninger, 1986), morphine (Olmstead and Franklin, 1997b), and more recently, ethanol (Walker and Ettenberg, 2001). Moreover, the ICV barbital place preference was produced at approximately 1/30 of the dose required systemically, which is comparable to the dose ratio for ICV (50 μg) (Morency and Beninger, 1986) and systemic (1 mg/kg) (Bedingfield et al., 1998) cocaine. It was not possible to test a higher ICV dose of barbital than 480 μg, since the concentration used was at its saturation point for a neutral pH. However, it is predicted that a higher dose would not induce a CPP and a full dose-response curve for the ICV place preference would resemble the systemic pentobarbital and barbital curves (i.e. inverted U-shaped).

Taken together, these findings demonstrate that, like other drugs of abuse, the reinforcing effects of barbiturates in the CPP task are centrally mediated. The fact that barbital is suitable for intracranial injections provides a methodology for the investigation of the neuropharmacological mechanisms of barbiturate reinforcement. With this methodology, the next chapter will examine the effects of administration of barbital into brain sites that are reported to be reinforcing in other drugs of abuse.

CHAPTER 5:

LOCALIZING THE REINFORCING PROPERTIES OF SODIUM BARBITAL: EFFECTS OF INTRACEREBRAL ADMINISTRATION

As discussed in Chapter 1, the reinforcing effects of drugs of abuse are thought to be mediated by the mesocorticolimbic DA system and extended amygdala. The areas involved in psychostimulant reinforcement are the NAc, medial prefrontal cortex, and ventral pallidum, and the areas involved in opiate reinforcement are the VTA and PAG. Because the posterior VTA appears to mediate the reinforcing effects of $GABA_A$ receptor agonists and ethanol, it is possible that the posterior VTA also mediates the reinforcing effects of barbiturates. It is also possible that areas that mediate the anxiolytic effects of GABAergic agents and other drugs may be involved in barbiturate reinforcement. These include the posterior hypothalamus (PH), PAG, and amygdala.

Blockade of GABA transmission by infusion of bicuculline methiodide or picrotoxin into the PH of rats elicits a pattern of physiological and behavioural arousal characterized by an increase in heart rate, increase in locomotor activity suggestive of an escape response, and pro-conflict behaviour (Shekhar and DiMicco, 1987; Shekhar et al., 1990). Conversely, facilitation of GABA transmission by infusion of muscimol into the PH produces a significant anti-conflict effect (Shekhar et al., 1990). Moreover, intra-PH injections of bicuculline methiodide increased the avoidance, but not approach, response in rats trained on a Sidman shock avoidance schedule while intra-PH injections of muscimol decreased both the avoidance and approach responses (Shekhar et al., 1987). Taken together, these findings suggest that an inhibitory GABAergic system within the PH modulates a "fight-or-flight" reaction. Activation of this system is anxiolytic while blockade of this system produces physiological arousal and is anxiogenic.

The PAG also appears to mediate anti-aversive effects of GABAergic drugs.

Microinjections of the $GABA_A$ receptor modulators chlordiazepoxide and midazolam into the PAG raised the current threshold that induces escape behaviour produced by electrical stimulation of the PAG, while local pretreatment with the benzodiazepine antagonist Ro 15-788 blocked this anti-aversive effect (Audi and Graeff, 1984). Local administration of GABA, muscimol, and pentobarbital into the PAG also increases the aversive threshold of PAG stimulation (Graeff et al., 1986b). Moreover, administration of picrotoxin and bicuculline into the PAG induces running and rearing behaviour which is similar to the effect of electrical stimulation of the PAG. Additionally, findings suggest that blocking GABAergic transmission in the PAG is aversive in the CPP paradigm. Microinjections of semicarbazide (6 μg), a GABA synthesis inhibitor, into the PAG induced a conditioned place aversion on test day and produced behavioural effects, such as attentive-like postures and rotational locomotion, during the conditioning phase (Di Scala and Sandner, 1989). Intra-PAG administration of muscimol (50 ng) significantly reduced the place aversion produced by semicarbazide, but had no effect when administered by itself. These findings suggest that in addition to the PH, the PAG may also mediate aversive behaviour, and that activation of the PAG GABAergic system may be involved in anti-aversive motivational states.

While a direct role of the amygdala in the reinforcing effects of drugs is not evident, the amygdaloid nuclear complex has been implicated in the neural basis of associative learning processes that are fundamental to incentive motivation (Everitt et al., 1999). With regard to GABAergic drugs, intra-amygdala injections of muscimol dose-dependently substitute for systemic administration of ethanol (Hodge and Cox, 1998) and

decrease ethanol self-administration in dependent rats (Roberts et al., 1996). Furthermore, injections of the competitive $GABA_A$ receptor antagonist SR 95531 into the central nucleus of the amygdala decrease responding for oral ethanol in a two-lever, free-choice task (Hyytia and Koob, 1995). The amygdala is also implicated in the anxiolytic effects of the positive $GABA_A$ modulator allopregnanolone. Akwa and colleagues (1999) reported that microinjections of the neurosteroid allopregnanolone into the central amygdala produced a significant increase in responding suppressed by punishment in the conflict test. Moreover, intra-central amygdala administration of allopregnanolone induced a significant increase in the time spent and the number of entries into the open arms in the elevated plus maze. Interestingly, other researchers have reported that systemic administration of allopregnanolone is reinforcing in rats and mice in the CPP paradigm (Finn et al., 1997; Franklin et al., 2002). Because the central amygdala appears to be involved in the anxiolytic-like actions of allopregnanolone (Akwa et al., 1999), and the lateral amygdala is reported to mediate some aspects of amphetamine place conditioning (Hiroi and White, 1991b), it is possible that different areas within the amygdala (e.g. lateral or central nucleus) may contribute to the reinforcing effects of barbital in the CPP paradigm.

The following experiment, therefore, tested whether barbital would be reinforcing in the place preference paradigm when administered into the PAG, PH, the lateral or central nucleus of the amygdala, or different regions (anterior or posterior) of the VTA.

5.1. METHODS

Animals

See Section 4.1.2.

Surgery

All surgical procedures were conducted under the same conditions as for Section 4.1.2. Permanent guide cannula were implanted bilaterally 1.5 mm (PH) or 2.0 mm (all other brain sites) above the intended site of injection according to the atlas of Paxinos and Watson (1998). The stereotaxic coordinates of the targeted sites are shown in Table 1.

Apparatus and Place Conditioning Procedure

The apparatus, conditioning procedure, and equipment used for intracerebral injections were the same as for Section 4.1.2.

Barbital was dissolved as in the experiments in Section 4.1. Rats were randomly assigned to a control group (0 μg) or 1, 5, 9, 15, or 25 μg barbital (see Table 1). The largest number of doses was examined in the intra-PAG CPP because it was the first brain area tested. The range of doses was subsequently adjusted for the other brain sites. Barbital was injected bilaterally at a rate of 0.5 μl/minute for 1 minute (1 μl total volume).

Histology

Euthanasia, perfusion, and brain sectioning was the same as for Section 4.1.2.

CNS Site	AP	L	V	Dose Barbital (µg)
CeA	−2.3	4.2	8.0	9, 15, 25
LA	−3.3	5.2	8.0	15, 25
PAG[a]	−6.0	1.8	6.0	1, 5, 15, 25
PAG (control)[a]	−6.0	1.8	4.5	25
PH[b]	−4.2	1.2	7.5	15, 25
antVTA[c]	−4.8	1.5	8.5	15, 25
postVTA[a]	−6.5	2.0	8.5	9, 15, 25

Table 1. Stereotaxic coordinates for intracerebral barbital injections

Values represent distances in millimeters, posterior (-) to bregma according to the atlas of Paxinos and Watson (1998). Abbreviations are CNS = central nervous system; AP = anterior/posterior; L = lateral; V = ventral (from skull); CeA = central amygdala; LA = lateral amygdala; PAG = periaqueductal gray; PH = posterior hypothalamus; antVTA = anterior ventral tegmental area; postVTA = posterior ventral tegmental area; µg = microgram

[a]Cannula angled laterally 10 degrees

[b]Cannula angled laterally 5 degrees

[c]Cannula angled laterally 6 degrees

Indian ink was not injected into the brain sites, since cannula tips were visible without the injection. Brains were stained with either Cresyl Violet or Formal Thionin (Fisher Scientific Inc). Data from individual subjects were discarded if bilateral injections were not symmetrical (less than 0.5 mm apart) or fell beyond the boundary of the target site.

Statistical Analysis

See Section 2.1.

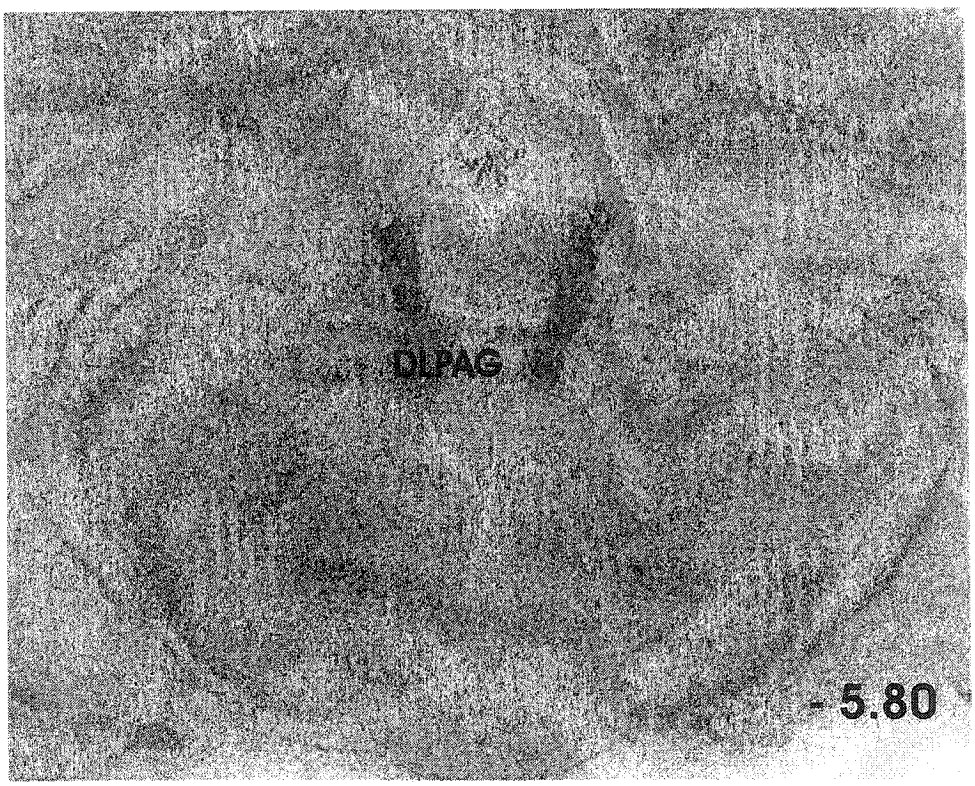
5.2. RESULTS

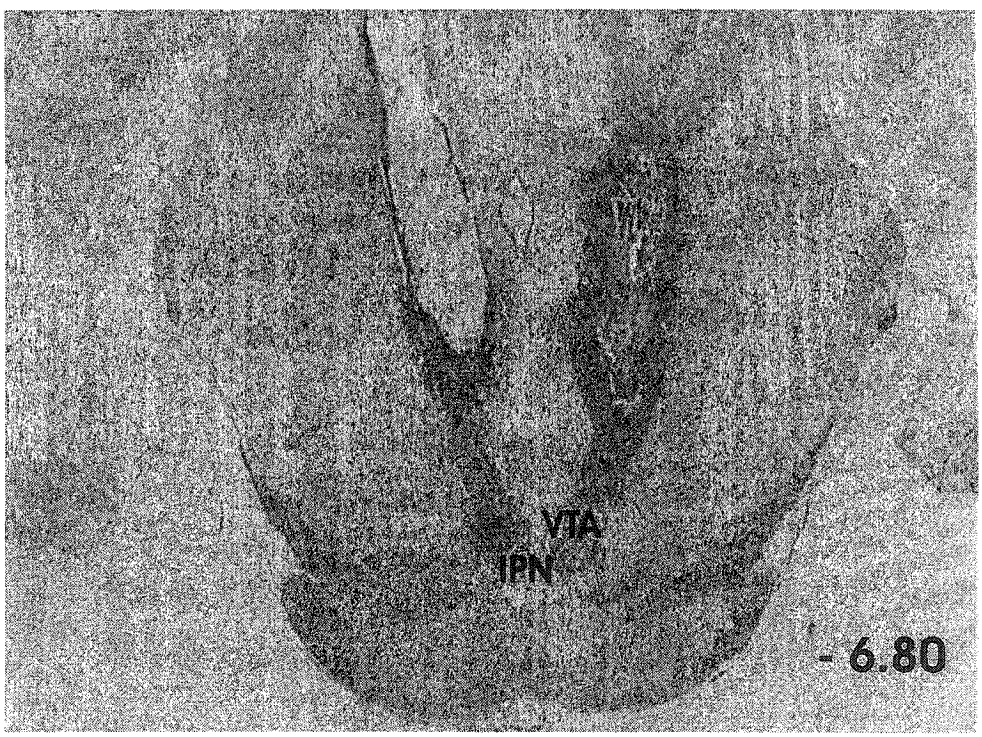
5.2.1. Intracerebral Place Preferences

Histology

Data from rats that had inaccurate or unilateral cannula placement were not used in the intracerebral place preference analyses. The number of animals eliminated from the groups were 10 from the PAG, 4 from the PH, 4 from the lateral amygdala, 6 from the central amygdala, 4 from the anterior VTA, and 7 from the posterior VTA. Of the 7 CNS sites tested, the only areas where barbital injections produced a CPP were the PAG and the posterior VTA. Figure 12 shows photomicrographs of the placements of microinjections into these two sites.

Figure 12. Photomicrographs depicting bilateral cannula placement in the periaqueductal gray (upper panel) and posterior ventral tegmental area (lower panel). Coordinates are posterior to bregma in millimeters. Abbreviations are DLPAG = dorsolateral periaqueductal gray; VTA = ventral tegmental area; IPN = interpeduncular nucleus


DLPAG
- 5.80


VTA
IPN
- 6.80

Intracerebral CPP

Microinjections of barbital into the PAG (25 μg) or posterior VTA (15 μg) induced a significant place preference, while microinjections of barbital into the PH, lateral amygdala, central amygdala, or anterior VTA did not induce a place preference or place aversion.

For the intra-PAG barbital place preference (n = 10/dose; Figure 13, upper panel), the only dose of barbital that produced a significant place preference was 25 μg ($F_{(1,45)}$ = 6.712, $p < 0.025$). 25 μg barbital injections 1.5 mm dorsal to the PAG did not produce a CPP ($t = 0.507$, $p = 0.628$, data not shown). No significant differences were obtained for locomotor activity (Figure 13, lower panel).

For the intra-posterior VTA place preference (n = 9-10/dose; Figure 14, upper panel), the rats spent significantly more time in the 15 μg barbital-paired compartment compared to the vehicle-paired compartment ($F_{(1, 36)} = 8.992$, $p < 0.01$). No significant differences were obtained for locomotor activity (Figure 14, lower panel).

Injections into all other sites (PH, lateral amygdala, central amygdala, or anterior VTA) were ineffective in producing a place preference (Figure 15 - 18, n = 8-10/dose).

5.2.2. Mapping the Intra-PAG and Intra-posterior VTA CPP

The findings from section 5.2.1. suggest that the PAG and the posterior VTA are involved in the reinforcing effects of the barbital-induced CPP. However, it is not certain whether particular subdivisions within the PAG or areas surrounding the posterior VTA

Figure 13. Upper panel: Mean time spent (seconds) in compartment paired with 0, 1, 5, 15, and 25 µg intra-periaqueductal gray (PAG) barbital (black bars) or vehicle (white bars). Lower panel: Mean activity counts in compartment paired with 0, 1, 5, 15, and 25 µg intra-PAG barbital (gray bars) or vehicle (white bars). *Vertical lines* mark the standard error of means (SEM). *Asterisks* indicate a significant difference ($p < 0.05$) between drug and saline condition.

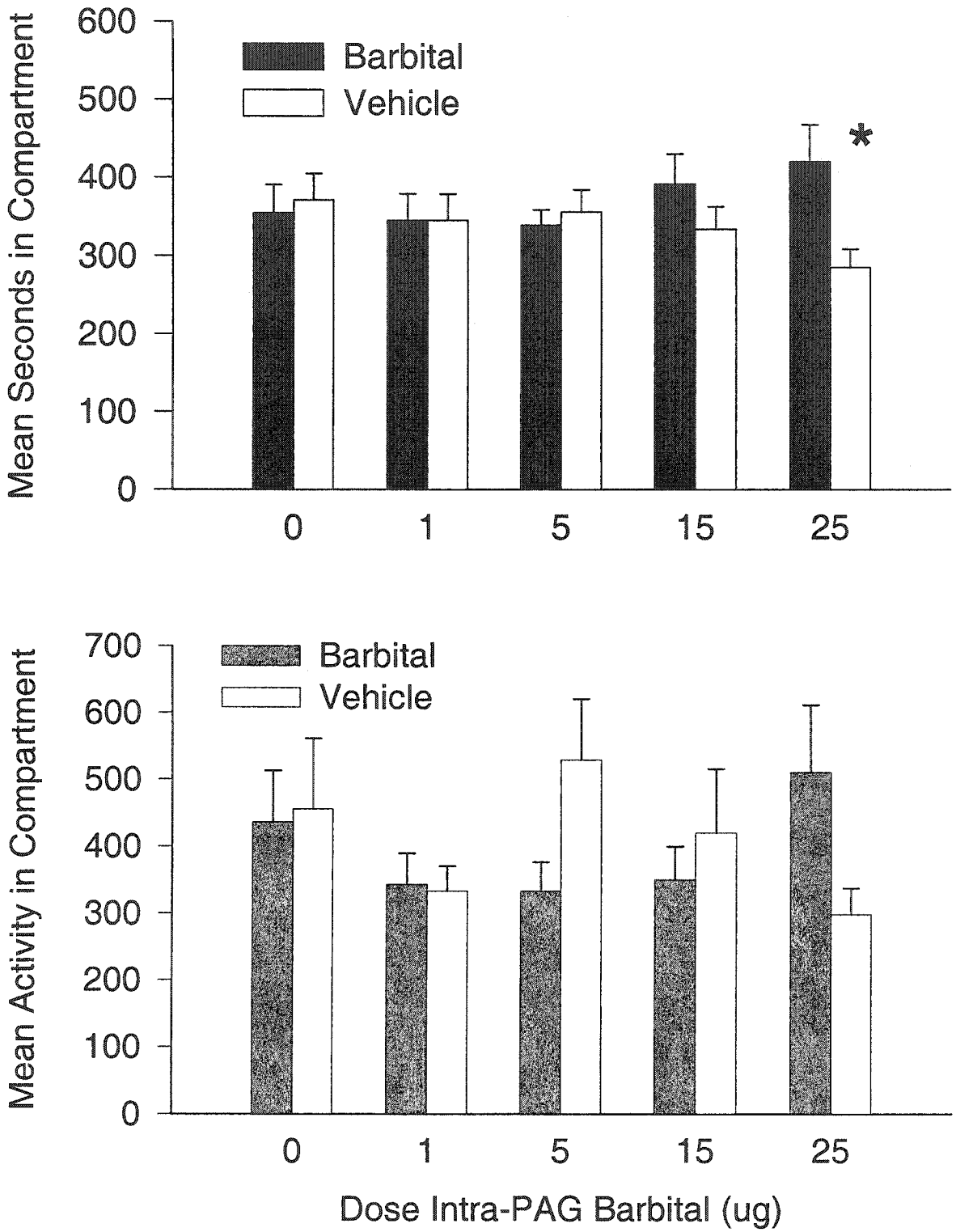
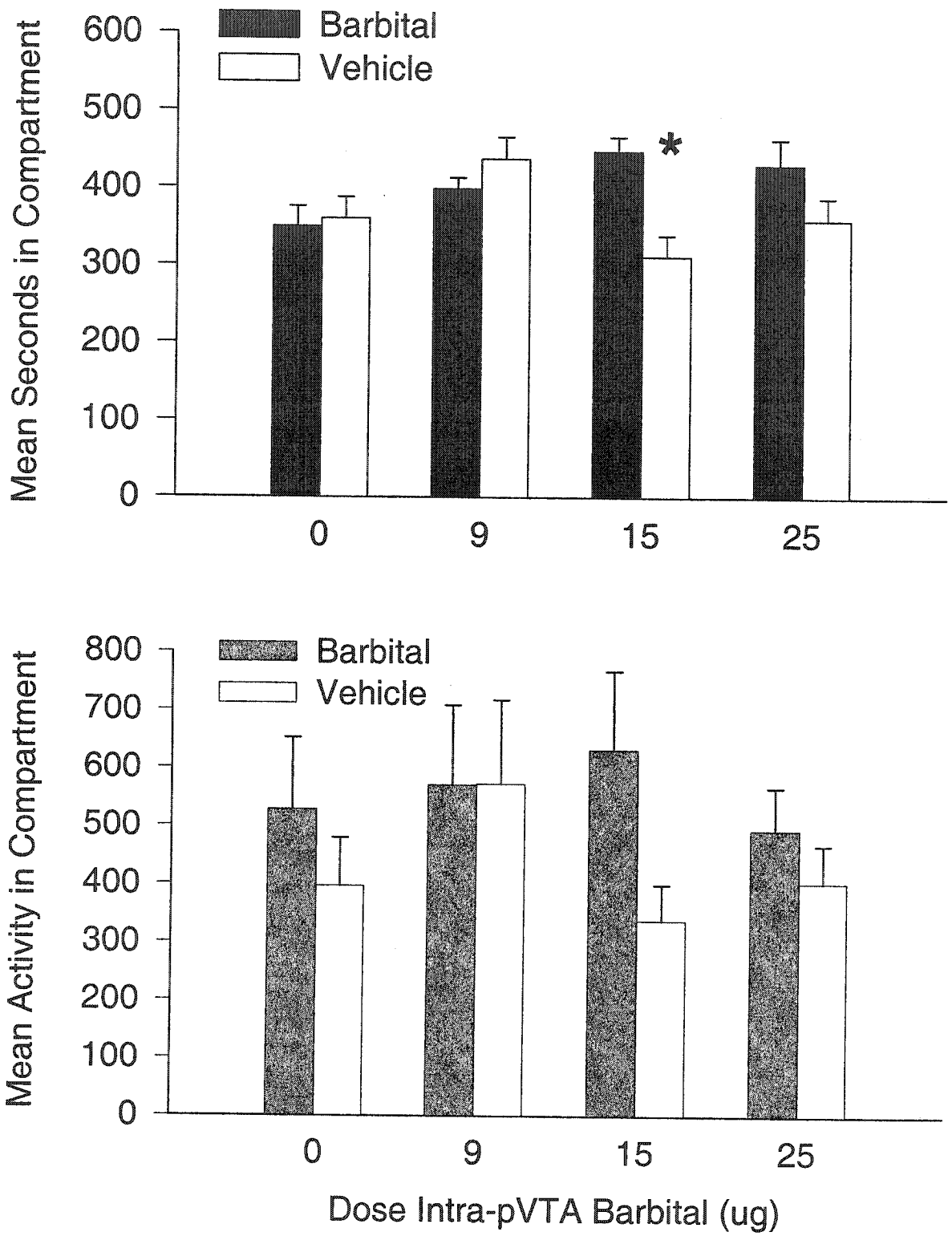

Mean Seconds in Compartment
600
500
400
300
200
100
0
Barbital
Vehicle
*
0
1
5
15
25
Mean Activity in Compartment
700
600
500
400
300
200
100
0
Barbital
Vehicle
0
1
5
15
25
Dose Intra-PAG Barbital (ug)

Figure 14. Upper panel: Mean time spent (seconds) in compartment paired with 0, 9, 15, and 25 µg intra-posterior ventral tegmental area (pVTA) barbital (black bars) or vehicle (white bars). Lower panel: Mean activity counts in compartment paired with 0, 9, 15, and 25 µg intra-pVTA barbital (gray bars) or vehicle (white bars). *Vertical lines* mark the standard error of means (SEM). *Asterisks* indicate a significant difference ($p < 0.05$) between drug and saline condition.


Barbital
Vehicle
Mean Seconds in Compartment
600
500
400
300
200
100
0
*
0
9
15
25
Barbital
Vehicle
Mean Activity in Compartment
800
700
600
500
400
300
200
100
0
0
9
15
25
Dose Intra-pVTA Barbital (ug)

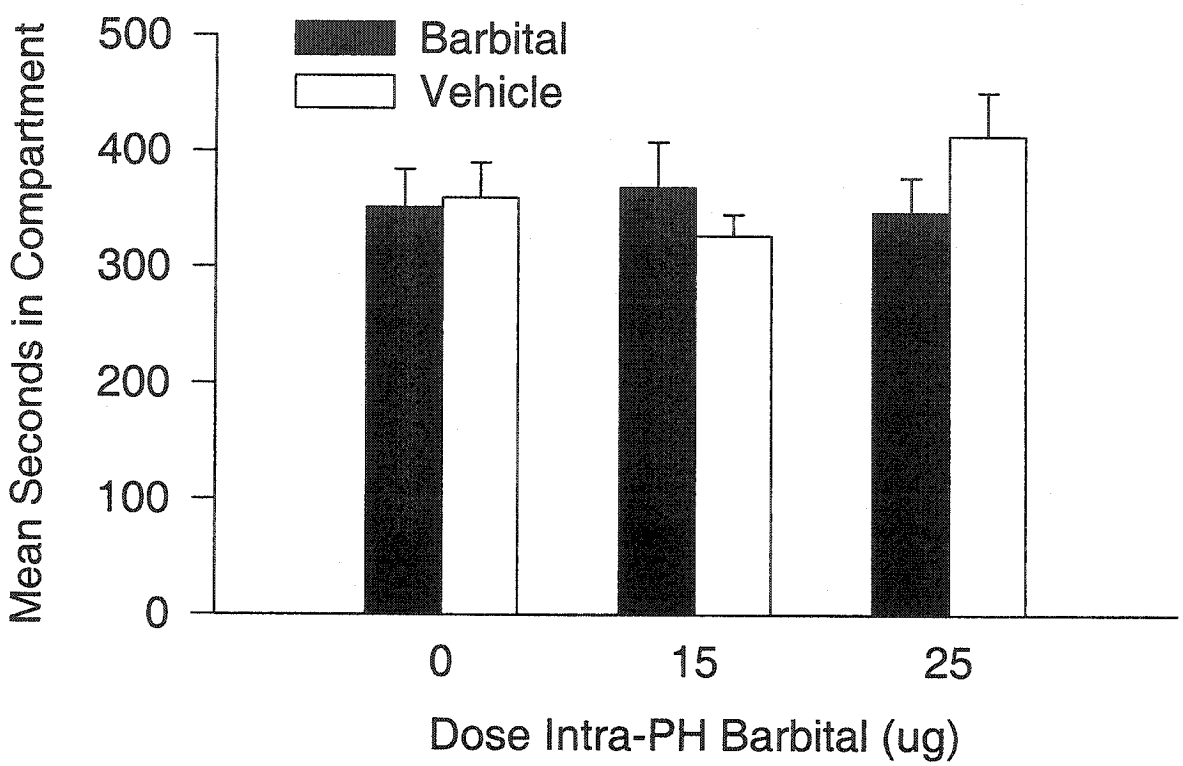


Figure 15. Mean time spent (seconds) in compartment paired with 0, 15, and 25 µg intra-posterior hypothalamus (PH) barbital (black bars) or vehicle (white bars). *Vertical lines* mark the standard error of the means (SEM). *Asterisks* indicate a significant difference ($p < 0.05$) between drug and vehicle condition.

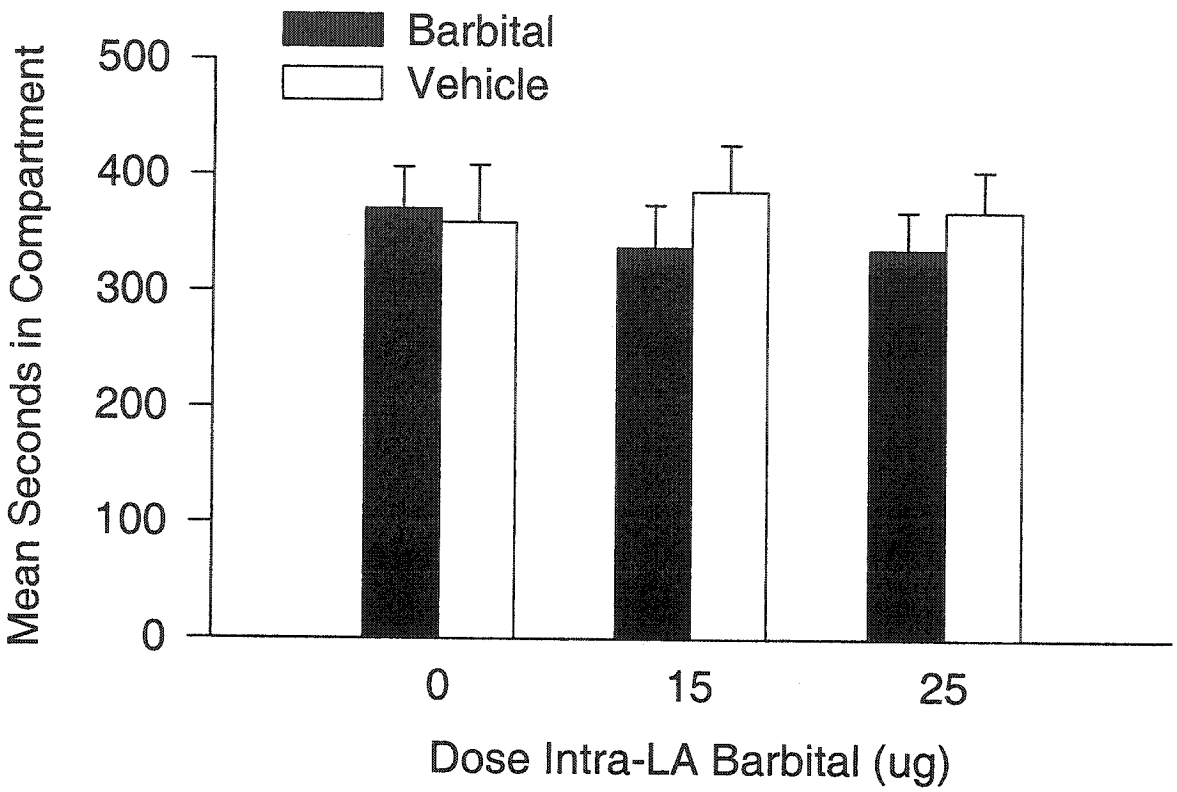


Figure 16. Mean time spent (seconds) in compartment paired with 0, 15, and 25 µg intra-lateral amygdala (LA) barbital (black bars) or vehicle (white bars). *Vertical lines* mark the standard error of the means (SEM). *Asterisks* indicate a significant difference ($p < 0.05$) between drug and vehicle condition.

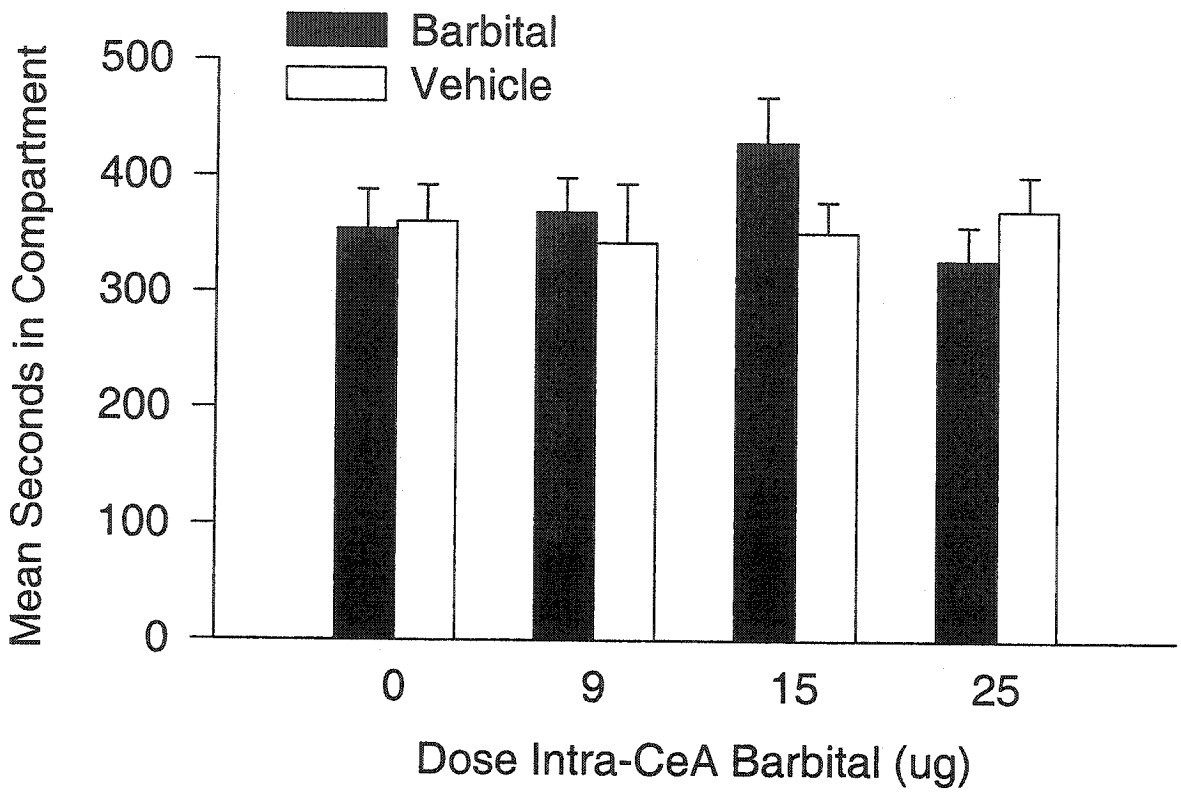


Figure 17. Mean time spent (seconds) in compartment paired 0, 9, 15, and 25 µg intra-central amygdala (CeA) barbital (black bars) or vehicle (white bars). *Vertical lines* mark the standard error of the means (SEM). *Asterisks* indicate a significant difference ($p < 0.05$) between drug and vehicle condition.

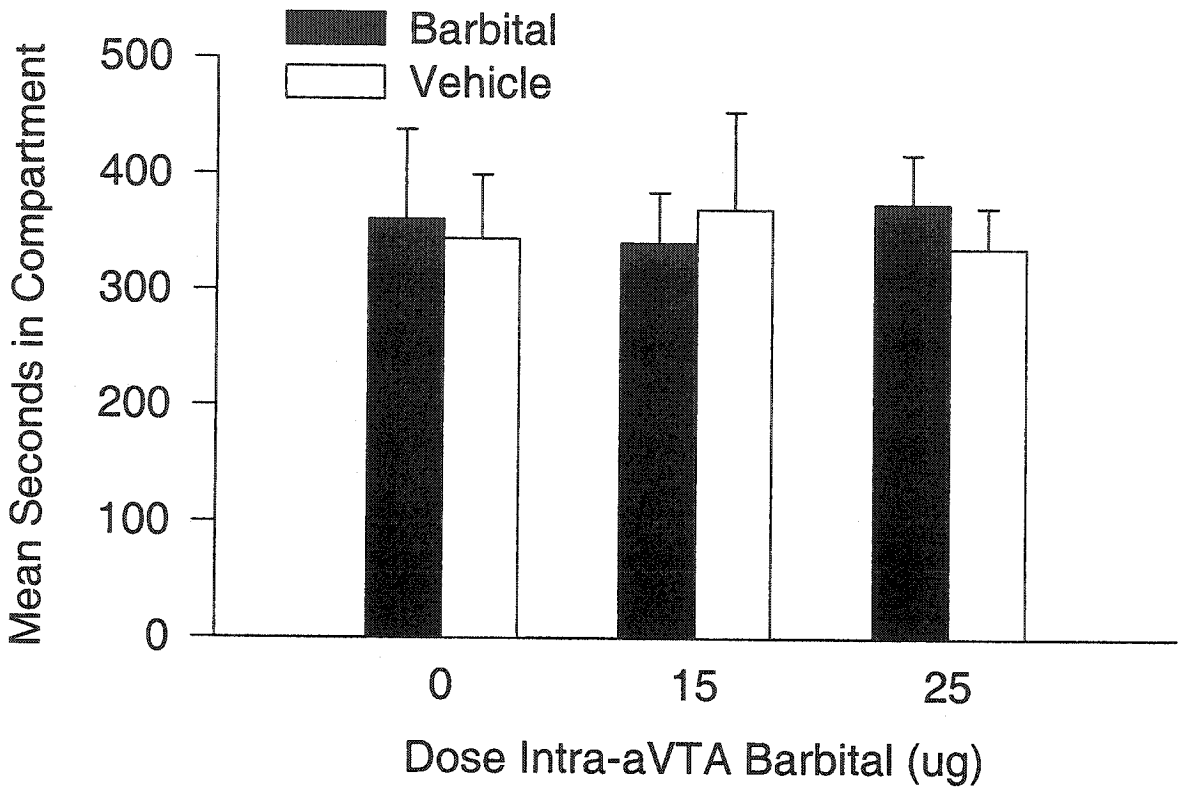


Figure 18. Mean time spent (seconds) in compartment paired with 0, 15, and 25 µg intra-anterior ventral tegmental area (aVTA) barbital (black bars) or vehicle (white bars). *Vertical lines* mark the standard error of the means (SEM). *Asterisks* indicate a significant difference ($p < 0.05$) between drug and vehicle condition.

are involved in the reinforcement properties of barbital. To explore this, microinjection sites from all of the 25 μg intra-PAG and 15 μg intra-pVTA rats (n=14/group) were included in the mapping study, and the CPP data from each brain area were separately analyzed. Mean difference scores (time spent in barbital-paired compartment minus time spent in vehicle-paired compartment) were calculated and classified according to the following criterion: Animals with a difference score greater than or equal to the median difference score were classified as having a strong preference (PREF), animals with a difference score greater than zero, but less than the median difference, were classified as having a moderate preference (MPREF), and animals with a difference score less than zero were classified as having no preference (NPREF). These 6 groups (PAG PREF, MPREF, or NPREF and pVTA PREF, MPREF, or NPREF) were then mapped onto their microinjection cannula sites using a stereotaxic atlas.

Figure 19 displays the microinjection sites for the PAG groups. All cannula sites were located between −5.6 and −6.3 mm posterior to bregma. Most of the PREF and MPREF sites are located within the dorsal and lateral divisions of the PAG. Injection sites that did not induce a preference (NPREF) appear to be located dorsal (i.e. within areas of the ventral superior colliculus) or lateral to the PAG. The median difference score for the PAG (25 μg) was 25.5 seconds (sec). The mean and standard deviation of difference scores for the groups were 245.85 ± 167.44 sec for PREF (n=7), 15.5 ± 7.78 sec for MPREF (n = 2), and −135.6 ± 106.88 sec for NPREF (n=5).

Figure 20 displays the microinjection sites for the pVTA groups. All cannula sites were located between −6.3 and −7.0 mm posterior to bregma. Most of the PREF and

Figure 19. Microinjection sites are plotted for intra-periaqueductal gray 25 µg barbital place preference. Coordinates are posterior to bregma in millimeters. Tips of cannula that produced a preference (PREF), moderate preference (MPREF) or no preference (NPREF) are respectively indicated by dark circles, dark stars, or gray squares (see text for details). Abbreviations are DLPAG = dorsolateral periaqueductal gray; LPAG = lateral periaqueductal gray; SupColl = superior colliculus.

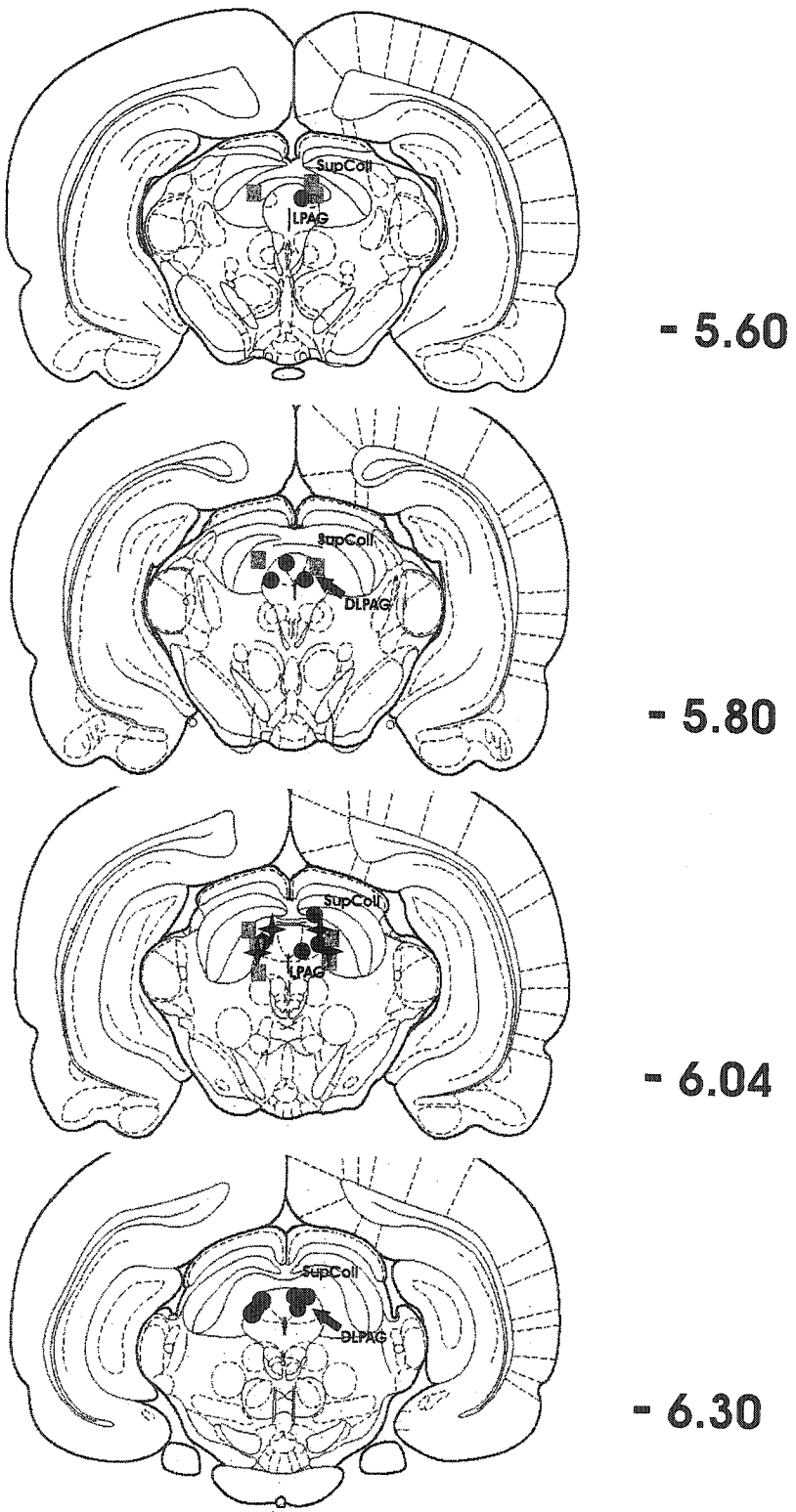
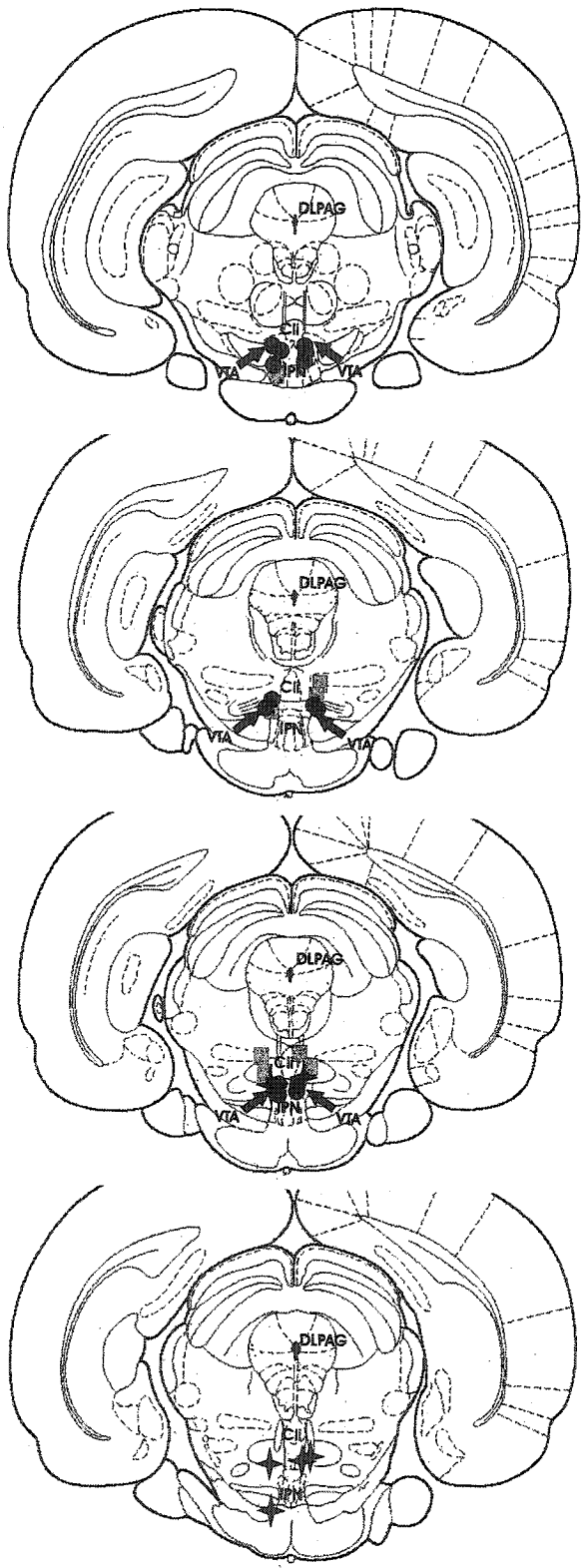

SupColl
LPAG
- 5.60
SupColl
DLPAG
- 5.80
SupColl
LPAG
- 6.04
SupColl
DLPAG
- 6.30

Figure 20. Microinjection sites are plotted for intra-posterior ventral tegmental area 15 µg barbital place preference. Coordinates are posterior to bregma in millimeters. Tips of cannula that produced a preference (PREF), moderate preference (MPREF) or no preference (NPREF) are respectively indicated by dark circles, dark stars, or gray squares (see text for details). Abbreviations are Cli = caudal linear nucleus; IPN = interpeduncular nucleus; VTA = ventral tegmental area.


DLPAG
CIi
VTA
IPN
VTA
- 6.30
DLPAG
CIi
VTA
IPN
VTA
- 6.72
DLPAG
CIi
VTA
IPN
VTA
- 6.80
DLPAG
CIi
IPN
- 7.04

MPREF sites are located within the posterior VTA and immediate surrounding areas (i.e. interpeduncular nucleus). Injection sites that did not induce a preference (NPREF) appear to be located dorsal to the posterior VTA (i.e. within the caudal linear nucleus). The median difference score for the pVTA (15 μg) was 125.5 sec. The mean and standard deviation of difference scores for the groups were 190.57 ± 46.0 sec for PREF (n=7), 79.67 ± 39.72 sec for MPREF (n = 3), and −125.25 ± 79.58 sec for NPREF (n=4).

5.3. DISCUSSION

Intra-PAG injections of 25 μg barbital, but not 1, 5, or 15 μg, induced a significant place preference. It is possible that higher concentrations of barbital administered into the PAG would have also been reinforcing, but saturation of barbital solution at a neutral pH prevented testing a higher concentration without increasing the intracerebral volume of injection beyond 1 μl. Analysis of the microinjection sites revealed that the animals that were in the PREF or MPREF groups all had cannula tips within the dorsolateral PAG. In contrast, the rats that were in the NPREF category had microinjection sites dorsal or lateral to the PAG (within the ventral superior colliculus). This is consistent with the control PAG group in which cannula that were 1.5 mm above the PAG did not produce a 25 μg place preference. The present finding suggests that the PAG is involved in the reinforcing effect of barbital in the CPP task. Likewise, the PAG is reported to be involved in the reinforcing effects of opiates. Mice self-administer morphine into the PAG (Cazala, 1990; David and Cazala, 1994b), and intra-PAG

injections of morphine produce a significant place preference in rats (van der Kooy et al., 1982; Olmstead and Franklin, 1997b).

The present finding is also consistent with reports that intra-PAG microinjections of drugs that facilitate GABAergic transmission are anti-aversive (Audi and Graeff, 1984; Graeff et al., 1986a; Graeff et al., 1986b), whereas intra-PAG microinjections of drugs that inhibit GABAergic transmission induce an aversive state (Di Scala and Sandner, 1989). Di Scala and Sandner (1989) argue that the fact that semicarbazide induces a place aversion supports the view that PAG neurons are tonically inhibited by GABA, and that disinhibition results in both the affective experience of aversion and the behavioural flight response. They further argue that aversive effects produced by PAG activation or disinhibition can support associative learning in Pavlovian paradigms. The fact that administration of the direct $GABA_A$ receptor agonist, muscimol (50 ng), into the PAG significantly attenuated the semicarbazide-induced place aversion demonstrates that the aversion is mediated specifically by disinhibition of GABAergic neurons. While Di Scala and Sandner did not find any evidence for the reinforcing effects of muscimol, it is possible that a higher dose (> 50 ng) is needed for muscimol to produce a place preference on its own.

Given the role of the PAG in anxiety, the question arises as to whether the intra-PAG barbital place preference may be the result of the anxiolytic or anti-aversive effects of barbital, rather than positive reinforcing effects of barbital. As discussed in the introduction of this chapter, the PH is also reported to mediate the aversive and anti-aversive effects of GABAergic drugs by administration of $GABA_A$ receptor antagonists

and agonists, respectively (Shekhar et al., 1990). If barbital produces effects that are anti-aversive or anxiolytic, then injections of barbital into the PH would also be expected to induce a CPP. However, injections of barbital into the PH did not produce a place preference. Moreover, although researchers acknowledge that a place preference could result from conditioning with anxiolytic drugs that reduce the putative aversive qualities of the CPP apparatus (Carr et al., 1989), evidence for such a reinforcing effect of anxiolytics in the place preference paradigm is not convincing. Some researchers report that administration of potent anxiolytics, such as benzodiazepines, induce a significant place preference (File, 1986; Acquas et al., 1989; Pain et al., 1997), while others do not (Pettit et al., 1989; Meririnne et al., 1999; Leri and Franklin, 2000b). Inconsistent findings also are reported for non-GABAergic anxiolytic drugs in the CPP task. While Neisewander and associates (1990) reported that buspirone and gespirone produced a place preference, others have reported that buspirone does not induce a significant CPP (File, 1986; Matsuzawa et al., 2000). Moreover, it was recently reported that using an unbiased paradigm, Ro 64-6198, an orphanin FQ receptor full agonist that has anxiolytic properties, also did not produce a place preference. Given these findings, it is unlikely that administration of barbital into the PAG induces a place preference because it is anxiolytic or anti-aversive.

The fact that barbital did not produce a place preference when administered into the PH is consistent with evidence that the PH is involved in a number of homeostatic mechanisms and defensive/escape behaviours, but not reinforcement. While rats demonstrate moderate levels of self-stimulation with electrodes implanted in the PH (Olds,

1956), microinjections of morphine into the PH does not produce a place preference (Olmstead and Franklin, 1997b).

Microinjections of barbital into the posterior, but not into the anterior, VTA produced a place preference. The dose that produced a CPP in the posterior VTA (15 μg) was lower than the dose that produced a CPP in the PAG (25 μg). This finding suggests that the posterior VTA is more sensitive to the reinforcing effects of barbital than the PAG. Analysis of microinjection sites indicate that the animals in the PREF or MPREF groups all had cannula tips within the posterior VTA or immediate surrounding areas, such as the interpeduncular nucleus. Cannula tips that were located dorsal to the posterior VTA, such as the caudal linear nucleus, did not induce a place preference (NPREF). This finding rules out the possibility that the intra-PAG CPP is because of diffusion of barbital from the posterior VTA to the PAG.

The intra-posterior VTA barbital place preference finding is consistent with recent reports that there are regional differences of $GABA_A$ receptor mediation within the VTA. $GABA_A$ receptor antagonists are self-administered into the anterior, but not the posterior, VTA, whereas $GABA_A$ receptor agonists are self-administered into the posterior, but not the anterior, VTA (Ikemoto et al., 1997b; Ikemoto et al., 1998). Ethanol is also self-administered into the posterior, but not the anterior, VTA (Rodd-Henricks et al., 2000), and $GABA_A$ antagonists, such as picrotoxin and bicuculline, decrease systemic self-administration of ethanol when injected into the anterior VTA (Nowak et al., 1998). Such decreases in self-administration suggest that the antagonists are promoting the effects of ethanol, since less drug is needed to maintain operant reinforcement. Furthermore,

Laviolette and van der Kooy (2001) reported that intra-VTA administration of muscimol (5 and 50 ng) induced a conditioned place preference. The finding that intra-VTA administration of barbital produced a CPP provides further evidence that drugs that facilitate $GABA_A$ receptor transmission are reinforcing when administered into the VTA. Moreover, it appears that the posterior, but not the anterior VTA, is more likely to mediate the reinforcing effects of barbiturates.

Because some of the cannula tips that were located in the interpeduncular nucleus also produced a place preference, it is possible that the interpeduncular nucleus, not the posterior VTA, mediates the barbital-induced place preference. Indeed, the interpeduncular nucleus contains high concentrations of GABA (Elekes et al., 1986) and GABA fibers are reported to be abundant in this area (Franzoni and Morino, 1989; Veenman and Reiner, 1994). However, while injections of the opioid agonist, DPDPE, into either the VTA or interpeduncular nucleus produced elevations in DA and DOPAC concentrations within the NAc, injections into the VTA were effective at doses lower than were injections into the interpeduncular nucleus (Devine et al., 1993). Given that the reinforcing effects of barbital are presumed to activate $GABA_A$ receptors located on GABA interneurons that increase VTA DA cell firing, it is unlikely that the interpeduncular nucleus plays a major role in the reinforcing effects of barbital.

Barbital did not produce a CPP when administered into the lateral or central nucleus of the amygdala. The amygdala is implicated in the reinforcing effects of a number of drugs of abuse in the self-administration paradigm, including amphetamine (Chevrette et al., 2002), morphine (David and Cazala, 1994a), and ethanol (Roberts et al.,

1996). Hiroi and White (1991b) reported that while electrolytic and neurotoxin lesions of the lateral amygdala prior to preconditioning only attenuated an amphetamine-induced CPP, lesions performed after conditioning (but before testing) completely blocked the effects of amphetamine. This finding suggests that the lateral nucleus of the amygdala mediates the expression of the amphetamine-induced CPP. However, place preferences are not produced by microinjections of amphetamine into either the central nucleus of the amygdala (Carr and White, 1986) or the lateral nucleus of the amygdala (Olmstead and Franklin, 1997b). Moreover, place preferences are also not obtained from microinjections of morphine into the amygdala (van der Kooy et al., 1982; Olmstead and Franklin, 1997b). Taken together, it appears that the amygdala does not play a primary role in the rewarding effects of the major drugs of abuse in the place preference paradigm.

The role of the NAc in psychostimulant-induced reward, and to a lesser degree in opiate reinforcement, is well established. The effects of administration of barbital into the NAc in the CPP task was not tested because there is very little evidence to support NAc-mediated reinforcement of GABAergic drugs. However, a recent study demonstrated that muscimol injections into the far rostral portion of the NAc shell produced a place preference, while injections into other regions of the NAc shell produced a place aversion (Reynolds and Berridge, 2002). Furthermore, injections of pentobarbital into the NAc are reported to substitute dose-dependently for systemic administration of ethanol (Hodge et al., 2001b), and intra-NAc shell administration of the DA receptor antagonist, fluphenazine, attenuates the development of a CPP produced by ICV ethanol administration (Walker and Ettenberg, 2002). Evidence from these recent reports suggest

that the NAc shell (and perhaps regional differences within the shell) supports $GABA_A$ receptor-mediated reinforcement, and that blockade of DA transmission within the NAc shell prevents reinforcement produced by GABAergic drugs. Further research is needed to delineate the precise role of the NAc DA and GABA neurons in the reinforcing effects of drugs that facilitate GABAergic neurotransmission.

In summary, the reinforcing effects of barbital appear to be mediated by the PAG and posterior VTA, which are the same neural sites that mediate morphine and ethanol reinforcement. However, these results do not indicate which neurochemical systems within these brain sites may contribute to the reinforcing effects of barbital in the CPP task. The findings from Chapter 3 suggest that the reinforcing effects of barbiturates are mediated by the GABAergic, dopaminergic, and opioid system, since antagonists from these neurochemical classes blocked the pentobarbital-induced CPP. The next section examines the possibility that antagonism of opioid or $GABA_A$ receptors in the PAG and/or in the posterior VTA will also block barbiturate reinforcement.

CHAPTER 6:

EFFECTS OF INTRACEREBRAL ADMINISTRATION OF OPIOID AND GABA ANTAGONISTS ON SODIUM BARBITAL-INDUCED PLACE PREFERENCE

If barbiturates use the same mechanisms as other drugs, then the reinforcing effects of barbiturates might be expected to depend on the same sites that mediate other drugs of abuse. As shown in Chapter 5, injections of barbital into the PAG and posterior VTA induced a significant place preference. This finding is consistent with reports that the PAG and posterior VTA mediate the reinforcing effects of opiate drugs (van der Kooy et al., 1982; Cazala, 1990; David and Cazala, 1994b; Olmstead and Franklin, 1997b), $GABA_A$ receptor agonists (Ikemoto et al., 1998), and ethanol (Rodd-Henricks et al., 2000). Moreover, it was shown in Chapter 3 that systemic administration of opioid and $GABA_A$ receptor antagonists block the systemically-induced pentobarbital CPP. Because the opioid system in the PAG and VTA is involved with opiate reinforcement, and the GABAergic system is involved with ethanol reinforcement, it is likely that opioid and GABAergic systems in the PAG and posterior VTA are also involved with barbital reinforcement.

It is well established that the dorsal PAG in the mesencephalon is a key structure in the integration of emotional reactions, defensive behaviours, and protective reactions to painful or aversive stimuli (Carrive, 1993; Behbehani, 1995). Furthermore, an opioid system appears to be an important modulator of these behaviours (Basbaum and Fields, 1978). Intra-dorsal PAG administration of morphine (< 30 nmol) is anti-aversive, assessed by an increase in the number of entries and time spent in the open arms of the elevated plus-maze (Motta and Brandao, 1993; Anseloni et al., 1999). Moreover, microinjections of morphine into the PAG produce long-lasting analgesia that is blocked by naloxone (Yaksh and Rudy, 1978), and microinjections of naloxone methobromide into

the PAG attenuates the analgesia produced by systemic administration of morphine (Manning and Franklin, 1998).

There is also evidence that the PAG may be involved in positive reinforcement. Self-administration of morphine into the PAG is blocked by systemic administration of naloxone (Cazala, 1990; David and Cazala, 1994b), and administration of methylnaltrexone into the PAG produces dose-related increases in heroin self-administration (Corrigall and Vaccarino, 1988). Furthermore, morphine administered into the PAG induces a significant place preference (van der Kooy et al., 1982; Olmstead and Franklin, 1997b), and intra-PAG injections of naloxone methiodide block a systemically-induced morphine place preference (Olmstead and Franklin, 1997b).

Another brain site that mediates opiate reinforcement is the VTA. Injections of morphine into the VTA significantly increases hypothalamic self-stimulation rates and decreases metencephalic self-stimulation rate-frequency thresholds (Broekkamp and Phillips, 1979; Rompre and Wise, 1989). Both of these effects are blocked by systemic administration of naloxone. Morphine and μ-receptor agonists are self-administered into the VTA (Bozarth and Wise, 1981; Devine and Wise, 1984; Zangen et al., 2002) and systemic administration of naloxone increases the rate of extinction produced by intra-VTA self-administration of morphine in mice (David and Cazala, 1994a). Furthermore, systemic or intra-VTA administration of naloxone blocks an intra-VTA- or systemic-induced morphine CPP, respectively (Phillips and LePiane, 1980; Olmstead and Franklin, 1997b). These findings indicate that activation of the mesolimbic reinforcement system is mediated, at least in part, by the opioid system.

Taken together, systemic or intracerebral (PAG or VTA) administration of opioid antagonists block the reinforcing effects of opioid agonists. Given that systemic administration of naloxone hydrochloride blocked a pentobarbital-induced CPP (see Chapter 3), it is possible that PAG or posterior VTA opioid mechanisms mediate the barbiturate CPP.

As previously discussed in Chapter 5, injections of $GABA_A$ receptor agonists into the PAG increase the aversive threshold of PAG stimulation (Graeff et al., 1986b) and in the VTA produce a conditioned place preference (Laviolette and van der Kooy, 2001), while intra-PAG administration of drugs that block GABAergic transmission into the PAG produces flight behaviour (Graeff et al., 1986b) and produces a place aversion (Di Scala and Sandner, 1989). Given this, it is possible that administration of a $GABA_A$ receptor antagonist into the PAG will block the induction of a barbital place preference. Moreover, as discussed in Chapter 1 (Section 1.1.3.) and Chapter 5, muscimol and ethanol are self-administered into the posterior VTA (Ikemoto et al., 1998; Rodd-Henricks et al., 2000), and co-infusion of $GABA_A$ antagonists with muscimol into the posterior VTA reduces the number of self-infusions (Ikemoto et al., 1998). If the reinforcing effects of barbital (see Chapter 4 and Chapter 5) are mediated by activation of $GABA_A$ receptors located on GABAergic interneurons that tonically inhibit VTA DA neurons, then local administration of a $GABA_A$ receptor antagonist into the posterior VTA should block its reinforcing effects.

Taken together, it was hypothesized that administration of an opioid receptor antagonist (naloxone methiodide) or a $GABA_A$ receptor antagonist (SR 95531) into either

the PAG or posterior VTA would interfere with a barbital-induced CPP. To avoid the cumulative effects of systemic injections of the long-acting barbital, barbital was administered ICV. Moreover, the $GABA_A$ receptor antagonist, SR 95531 (gabazine), rather than bicuculline, was used in these experiments. This is because recent reports indicate that SR 95531 is a more selective and specific $GABA_A$ receptor antagonist than bicuculline or its quaternary derivatives (Yu and Ho, 1990). Moreover, in addition to their $GABA_A$ receptor antagonist effects, bicuculline and its quaternary derivatives block the apamin-sensitive component of afterhyperpolarization in DA cells (Seutin et al., 1997) and potentiate calcium transients in rat cerebellar granule cells evoked by potassium chloride (voltage-gated) or A23187 (ionophore facilitated) (Mestdagh and Wulfert, 1999). The latter effect is not likely mediated by $GABA_A$ receptor antagonism, since SR 95531 decreased, rather than increased, potassium chloride-induced calcium transients.

6.1. METHODS

Animals

See Section 4.1.2

Surgery

All surgical procedures were conducted under the same conditions as described in Chapters 4 and 5. Each rat had cannula implanted bilaterally in the ventricles and cannula implanted bilaterally in either the PAG or posterior VTA. The stereotaxic coordinates of

the targeted sites are the same as in Section 5.1. (See Table 1).

Apparatus and Place Conditioning Procedure

The apparatus, conditioning procedure, and equipment used for intracerebral injections were the same as for Section 5.1. Injections into the two sites occurred sequentially. In other words, the rats were injected with the antagonist (or vehicle) into the PAG or posterior VTA and then injected with barbital (or vehicle) into the lateral ventricles. Immediately after the ICV injections, the rats were placed into the conditioning compartments.

6.1.1. Naloxone Methiodide Administered into the PAG or posterior VTA

Barbital and naloxone methiodide (NMI) were both prepared fresh daily. Preparation and ICV injection of barbital (480 μg) and vehicle (0 μg) was the same as in Section 4.1.2. NMI (Sigma) was dissolved in 0.9% physiological saline, and final doses were 0.5, 2.0, and 5.0 nmol (0.235, 0.94, and 2.35 μg). NMI or vehicle (saline) was injected bilaterally at a rate of 0.25 μl/minute for 1 minute (0.5 μl total volume). Rats received injections of 0.0, 0.5, 2.0, or 5.0 nmol NMI into the PAG or 0.0, 0.5, or 2.0 nmol NMI into the posterior VTA followed by administration of 480 μg ICV barbital. The highest dose of NMI administered into the PAG (5 nmol) was not tested in the posterior VTA. Additionally, different groups of rats were administered 2.0 nmol NMI into the PAG or posterior VTA followed by 0 μg barbital (i.e. vehicle) to test for potential effects

of NMI on its own.

6.1.2. SR 95531 Administered into the PAG or posterior VTA

Barbital was prepared and administered the same as in preceding experiment. SR 95531 (Sigma) was prepared fresh daily and was dissolved in 0.9% physiological saline to obtain final doses of 1.25, 2.5, and 5.0 ng. Volume and rate of injections of SR 95531 or vehicle (saline) was the same as NMI. Rats received injections of 0.0, 1.25, 2.5, or 5.0 ng SR 95531 into the PAG or 0.0, 2.5, or 5.0 ng SR 95531 into the posterior VTA followed by administration of 480 μg ICV barbital. The lowest dose of SR 95531 administered into the PAG (1.25 ng) was not tested in the posterior VTA. Additionally, different groups of rats were administered 2.5 (PAG) or 5.0 ng (posterior VTA) SR 95531 followed by 0 μg barbital (i.e. vehicle) to test for potential effects of SR 95531 on its own.

Histology

See Section 5.1.

Statistical Analysis

See Section 2.1.

6.2. RESULTS

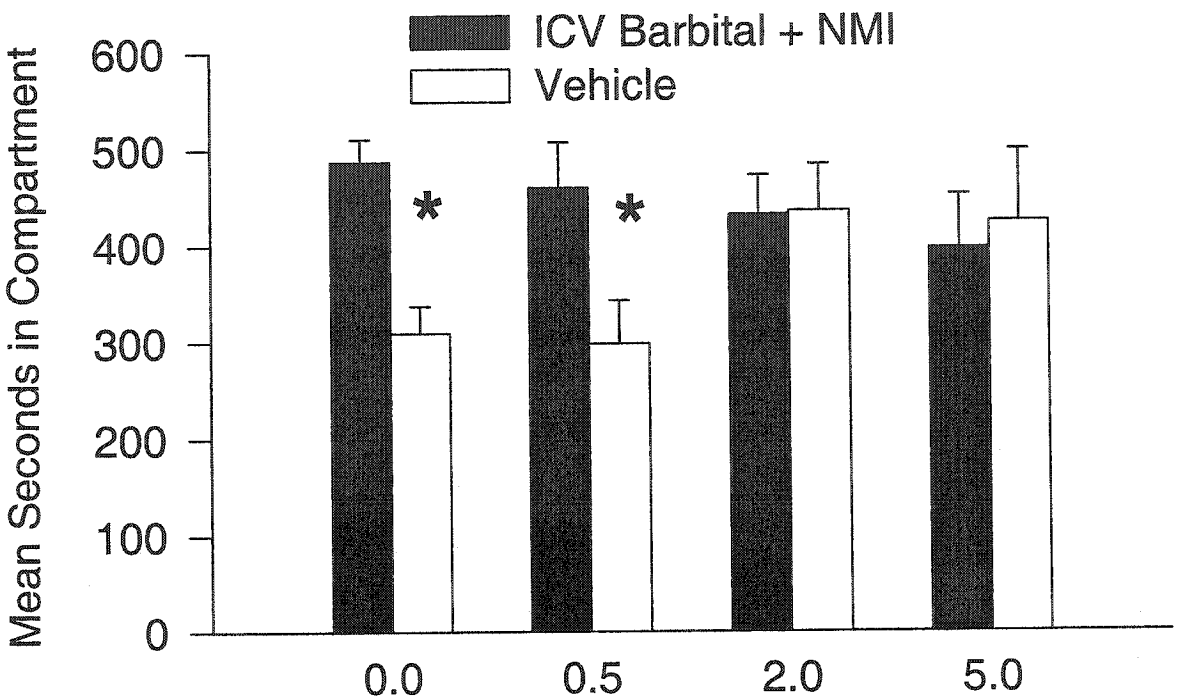
Histology

Data from rats that had inaccurate or unilateral cannula placement in the lateral ventricles or in either the PAG or posterior VTA were not used in the final analyses. The number of animals eliminated from the groups were 5 from the NMI-PAG group, 7 from the NMI-posterior VTA group, 6 from the SR 95531-PAG group, and 7 from the SR 95531-posterior VTA group. Additionally, 2 animals from each of the NMI-PAG group (5 nmol NMI) and SR 95531-PAG group (5 ng) were eliminated because they displayed intense defensive and escape behaviours following intra-PAG antagonist pretreatment.

Intra-PAG NMI + ICV Barbital CPP

As shown in Figure 21 (upper panel), compared to the vehicle-paired compartments, the rats spent significantly more time in the compartments paired with 480 μg ICV barbital plus vehicle ($F_{(1,25)} = 5.464$, $p < 0.05$), or with ICV barbital plus 0.5 nmol NMI ($F_{(1,25)} = 3.96$, $p < 0.05$) (n = 6-8/dose). Injections of 2.0 or 5.0 nmol NMI into the PAG blocked the ICV barbital place preference, because at these doses, the animals did not significantly differ in the time spent in the drug-paired or vehicle-paired compartments ($F_{(1,25)} = 0.002$, NS and $F_{(1,25)} = 0.99$, NS, respectively). In terms of locomotor activity, planned comparisons revealed that the rats were significantly more active in the compartment paired with ICV barbital plus vehicle ($F_{(1,25)} = 5.872$, $p < 0.025$), but not in the compartments paired with barbital plus any of the doses of NMI: 0.5 nmol ($F_{(1,25)} =$

Figure 21. Upper panel: Mean time spent (seconds) in compartment paired with 0, 0.5, 2.0, and 5.0 nmol intra-periaqueductal gray (PAG) naloxone methiodide (NMI) pretreatment to 480 µg ICV barbital (black bars) or vehicle (white bars). Lower panel: Mean activity counts in compartment paired with 0, 0.5, 2.0, and 5.0 nmol intra-PAG NMI pretreatment (gray bars) or vehicle (white bars). *Vertical lines* mark the standard error of the means (SEM). *Asterisks* indicate a significant difference ($p < 0.05$) between drug and vehicle condition.


ICV Barbital + NMI
Vehicle
Mean Seconds in Compartment
600
500
400
300
200
100
0
*
*
0.0
0.5
2.0
5.0

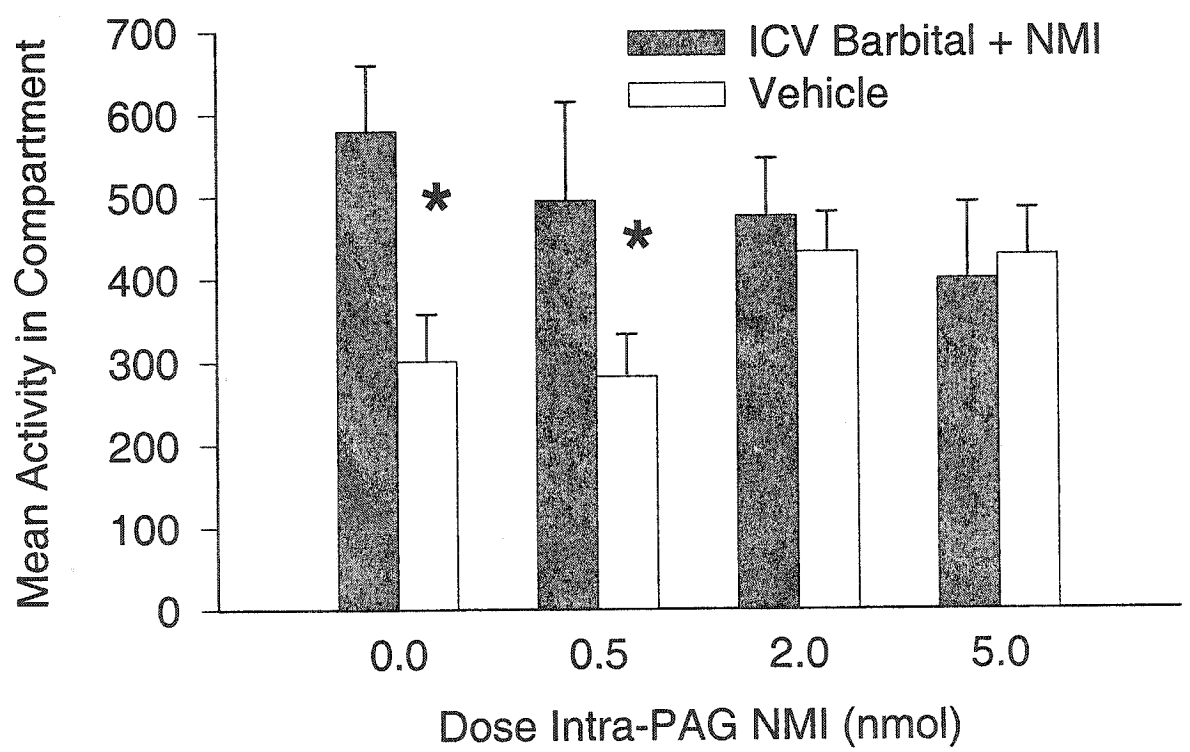
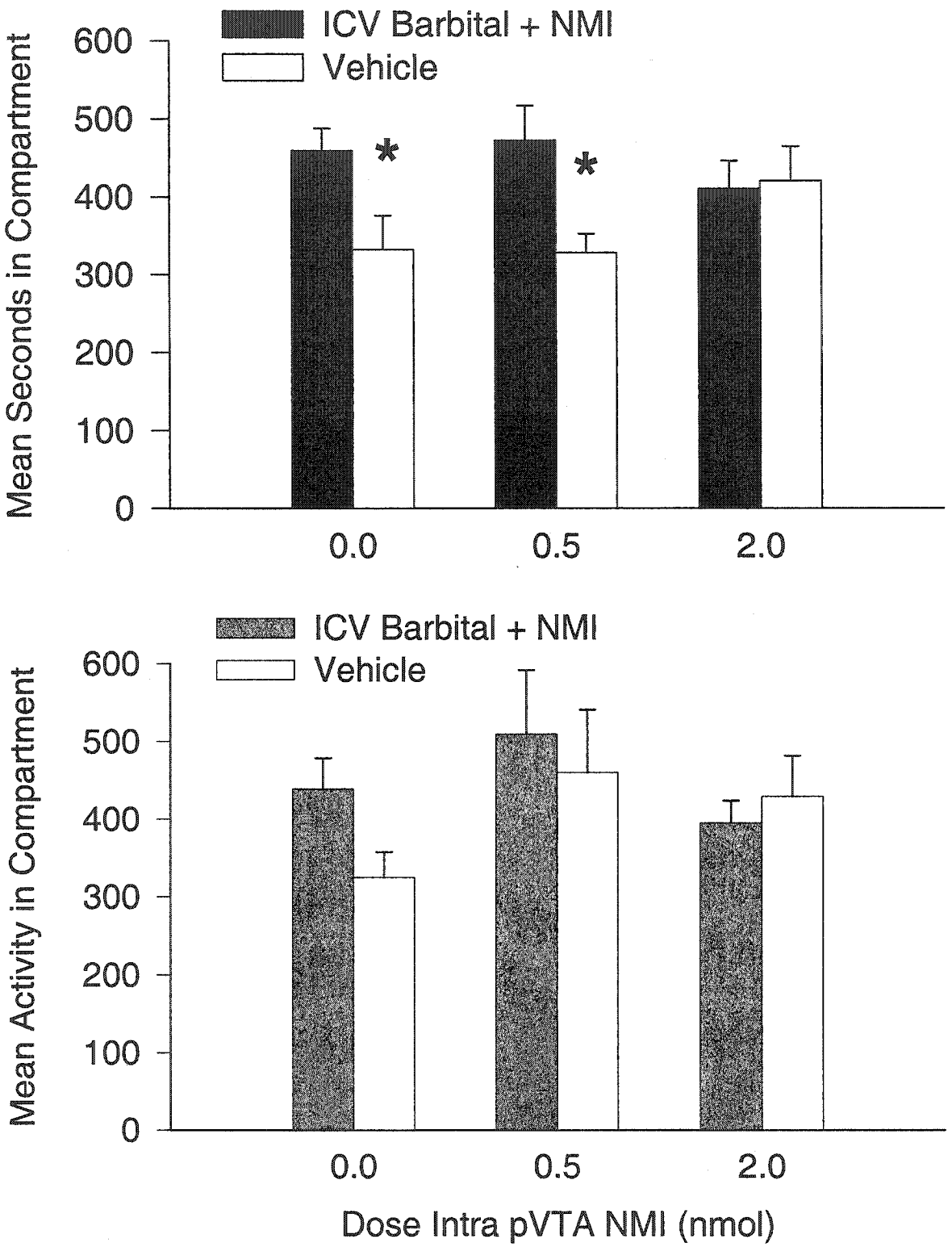

ICV Barbital + NMI
Vehicle
Mean Activity in Compartment
700
600
500
400
300
200
100
0
*
*
0.0
0.5
2.0
5.0
Dose Intra-PAG NMI (nmol)

Figure 22. Upper panel: Mean time spent (seconds) in compartment paired with 0, 0.5, and 2.0 nmol intra-posterior ventral tegmental area (pVTA) naloxone methiodide (NMI) pretreatment to 480 µg ICV barbital (black bars) or vehicle (white bars). Lower panel: Mean activity counts in compartment paired with 0, 0.5, and 2.0 nmol intra-pVTA NMI pretreatment (gray bars) or vehicle (white bars). *Vertical lines* mark the standard error of the means (SEM). *Asterisks* indicate a significant difference ($p < 0.05$) between drug and vehicle condition.


ICV Barbital + NMI
Vehicle
Mean Seconds in Compartment
600
500
400
300
200
100
0
*
*
0.0
0.5
2.0
ICV Barbital + NMI
Vehicle
Mean Activity in Compartment
600
500
400
300
200
100
0
0.0
0.5
2.0
Dose Intra pVTA NMI (nmol)

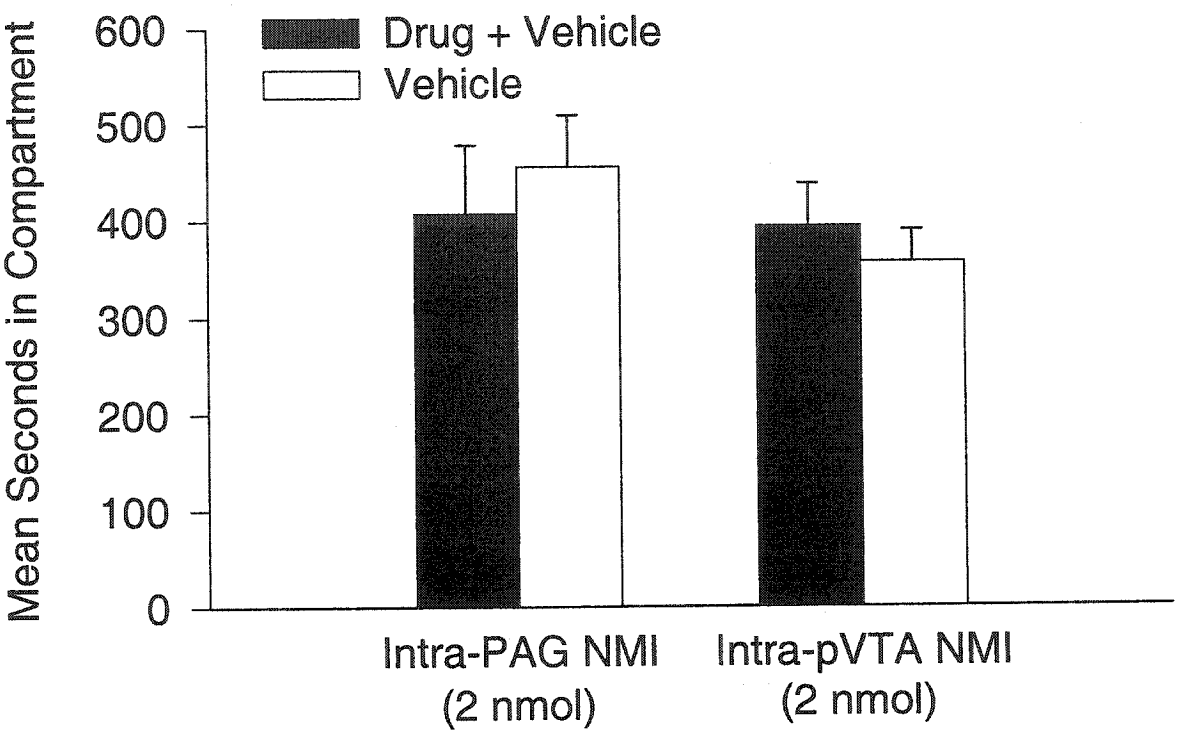


Figure 23. Mean time spent (seconds) in compartments paired with 2 nmol NMI intra-PAG or intra-pVTA pretreatment to vehicle (black bars) or vehicle pretreatment to barbital control (vehicle) (white bars). *Vertical lines* mark the standard error of the means (SEM). *Asterisks* indicate a significant difference ($p < 0.05$) between drug and vehicle condition.

2.985, NS), 2.0 nmol ($F_{(1,25)}$ = 0.147, NS), or 5.0 nmol ($F_{(1,25)}$ = 0.045, NS; Figure 21, lower panel).

NMI (2.0 nmol) administered into the PAG (n = 7) followed by ICV vehicle did not produce a place preference or aversion (t = -0.413, p = 0.694; Figure 23).

Intra-posterior VTA NMI + ICV Barbital CPP

As shown in Figure 22 (upper panel), similar to intra-PAG NMI, the rats spent significantly more time in the compartments paired with 480 μg ICV barbital plus vehicle ($F_{(1,21)}$ = 3.985, $p < 0.05$), or with ICV barbital plus 0.5 nmol NMI ($F_{(1,21)}$ = 5.133, $p < 0.05$) compared to the vehicle-paired compartments (n = 8/dose). The higher dose of NMI (2 nmol) blocked the ICV barbital place preference ($F_{(1,21)}$ = 0.023, NS). For locomotor activity, no significant within-subjects comparisons were found (Figure 22, lower panel).

NMI (2.0 nmol) administered into the posterior VTA (n = 8) followed by ICV vehicle did not produce a place preference or aversion (t = 0.508, p = 0.627; Figure 23).

Intra-PAG SR 95531 + ICV Barbital CPP

As shown in Figure 24 (upper panel), compared to the vehicle-paired compartments, the rats spent significantly more time in the compartments paired with 480 μg ICV barbital plus vehicle ($F_{(1,30)}$ = 3.468, $p < 0.05$), or with ICV barbital plus 1.25 ng SR 95531 ($F_{(1,25)}$ = 5.652, $p < 0.05$) (n = 6-10/dose). SR 95531 (2.5 and 5.0 ng) blocked the ICV barbital place preference ($F_{(1,30)}$ = 0.087, NS and $F_{(1,30)}$ = 0.128, NS, respectively).

Figure 24. Upper panel: Mean time spent (seconds) in compartment paired with 0, 1.25, 2.5, and 5.0 ng intra-PAG SR 95531 pretreatment to 480 µg ICV barbital (black bars) or vehicle (white bars). Lower panel: Mean activity counts in compartment paired with 0, 1.25, 2.5, and 5.0 ng intra-PAG SR 95531 pretreatment (gray bars) or vehicle (white bars). *Vertical lines* mark the standard error of the means (SEM). *Asterisks* indicate a significant difference ($p < 0.05$) between drug and vehicle condition.

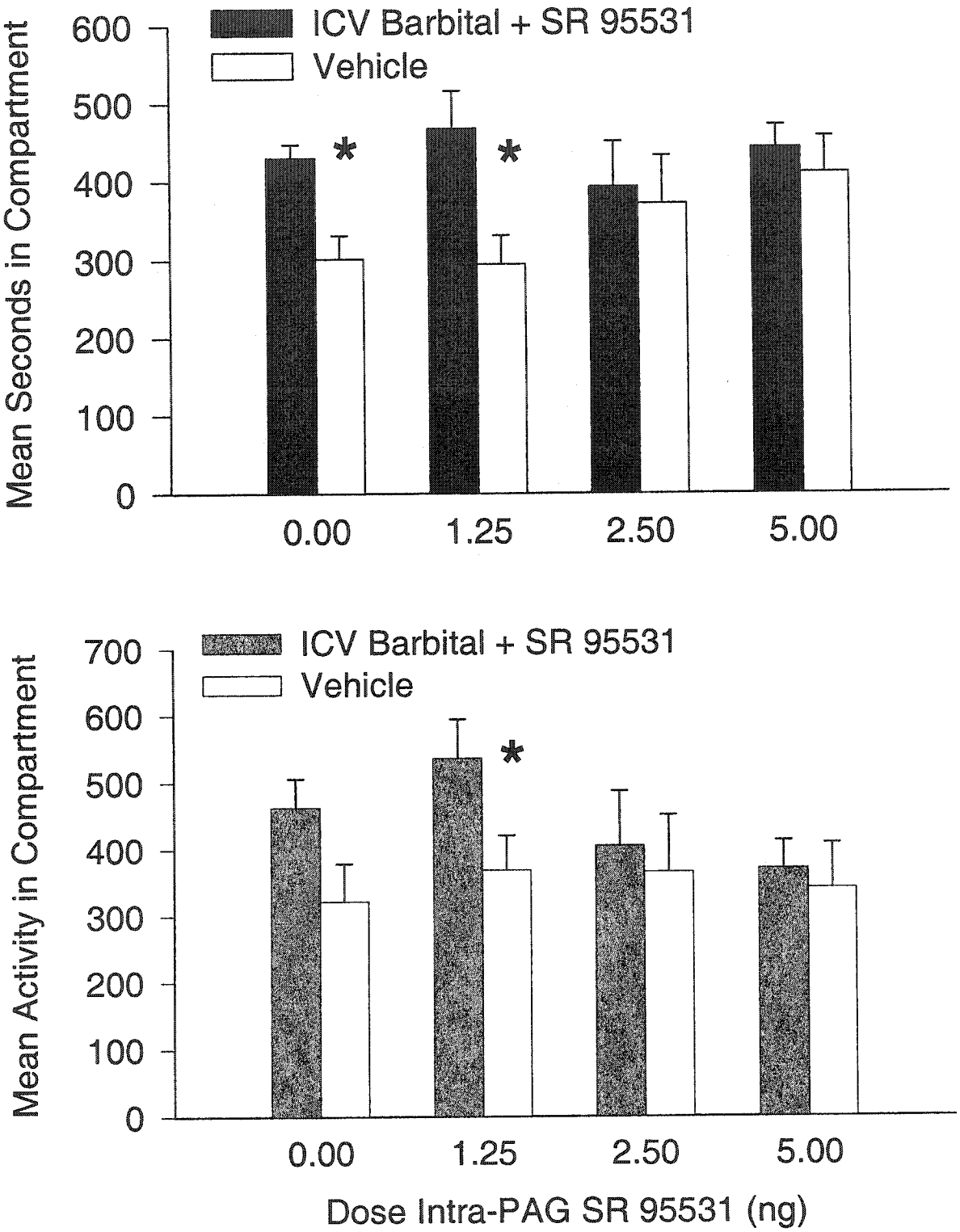
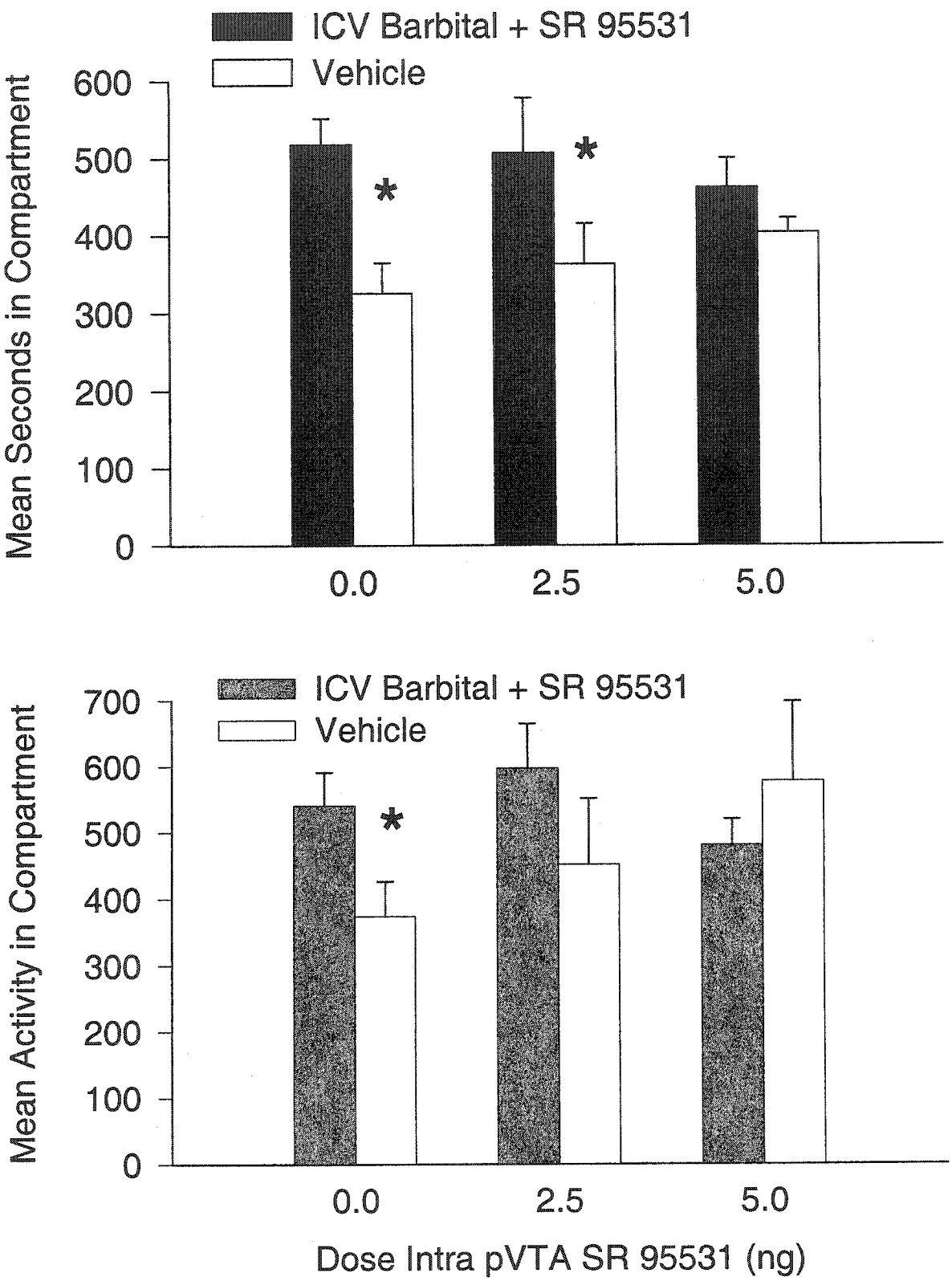

ICV Barbital + SR 95531
Vehicle
Mean Seconds in Compartment
600
500
400
300
200
100
0
*
*
0.00
1.25
2.50
5.00
ICV Barbital + SR 95531
Vehicle
Mean Activity in Compartment
700
600
500
400
300
200
100
0
*
0.00
1.25
2.50
5.00
Dose Intra-PAG SR 95531 (ng)

Figure 25. Upper panel: Mean time spent (seconds) in compartment paired with 0, 2.5, and 5.0 ng intra-pVTA SR 95531 pretreatment to 480 µg ICV barbital (black bars) or vehicle (white bars). Lower panel: Mean activity counts in compartment paired with 0, 2.5, and 5.0 ng intra-pVTA SR 95531 pretreatment (gray bars) or vehicle (white bars). *Vertical lines* mark the standard error of the means (SEM). *Asterisks* indicate a significant difference ($p < 0.05$) between drug and vehicle condition.


ICV Barbital + SR 95531
Vehicle
Mean Seconds in Compartment
600
500
400
300
200
100
0
*
*
0.0
2.5
5.0
ICV Barbital + SR 95531
Vehicle
Mean Activity in Compartment
700
600
500
400
300
200
100
0
*
0.0
2.5
5.0
Dose Intra pVTA SR 95531 (ng)

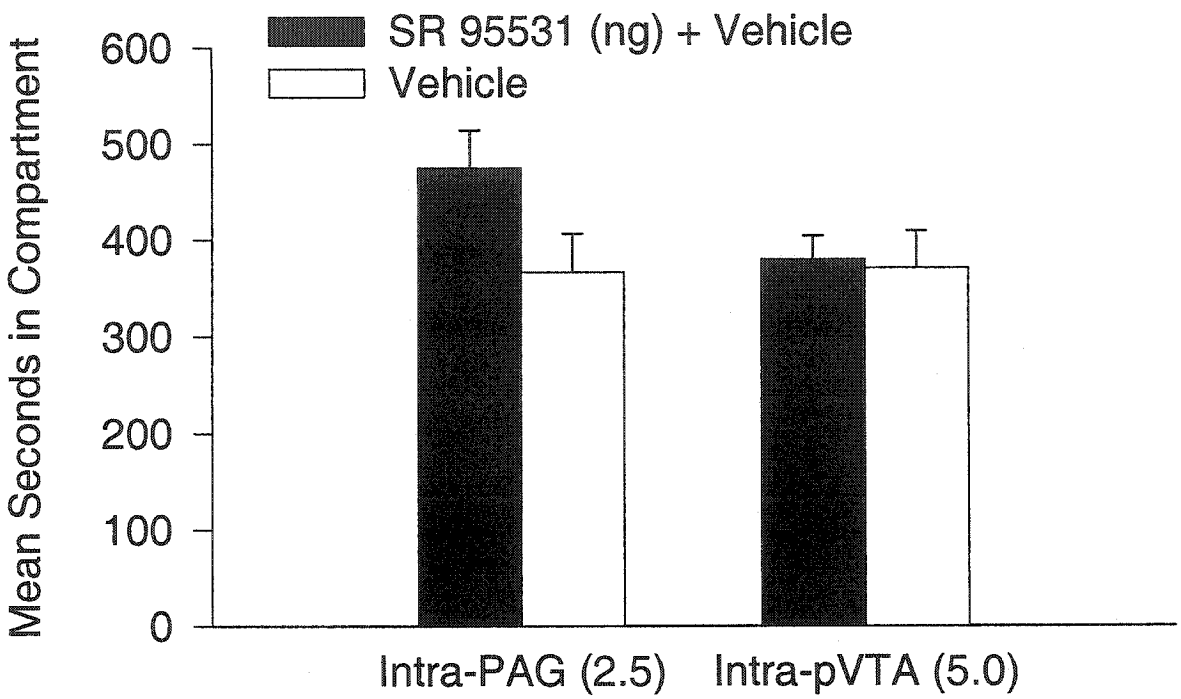


Figure 26. Mean time spent (seconds) in compartments paired with 2.5 ng (intra-PAG) or 5.0 ng (intra-pVTA) SR 95531 pretreatment to vehicle (black bars) or vehicle pretreatment to barbital control (vehicle) (white bars). *Vertical lines* mark the standard error of the means (SEM). *Asterisks* indicate a significant difference ($p < 0.05$) between drug and vehicle condition.

In terms of locomotor activity, planned comparisons revealed that the rats were significantly more active in the compartments paired with barbital plus 1.25 ng SR 95531 ($F_{(1,30)} = 4.457$, $p < 0.05$; Figure 24, lower panel).

SR 95531 (2.5 ng) administered into the PAG (n = 9) followed by ICV vehicle did not produce a place preference or aversion ($t = 1.390$, $p = 0.202$; Figure 26).

Intra-posterior VTA SR 95531 + ICV Barbital CPP

As shown in Figure 25 (upper panel), the rats spent significantly more time in the compartments paired with 480 μg ICV barbital plus vehicle ($F_{(1,24)} = 5.372$, $p < 0.05$) and ICV barbital plus 2.5 ng SR 95531 ($F_{(1,24)} = 3.017$, $p < 0.05$) (n = 9/dose). The higher dose of SR 95531 (5.0 ng) blocked the ICV barbital CPP ($F_{(1,24)} = 0.495$, NS). With regard to locomotor activity, no significant within-subjects comparisons were found (Figure 25, lower panel).

SR 95531 (5.0 ng) administered into the posterior VTA (n = 8) followed by ICV vehicle did not produce a place preference or aversion ($t = 0.160$, $p = 0.878$; Figure 26).

6.3. DISCUSSION

6.3.1. Naloxone Methiodide

The higher doses of NMI administered into the PAG or posterior VTA blocked the ICV barbital place preference. The lowest dose of NMI tested (0.5 nmol) did not block the place preference when administered into either area. These results indicate that the

PAG and posterior VTA are equally sensitive to opioid antagonism by NMI. Although injections of 5 nmol NMI into the PAG produced aversive-like defensive behaviour in two animals, the lower dose of NMI (2 nmol) that also blocked the ICV barbital CPP did not produce a place aversion when vehicle was administered into the ventricles. Likewise, NMI administered by itself into the posterior VTA also did not produce a place aversion. These findings indicate that NMI antagonism of ICV barbital place preference is not because of the aversive effects of the opioid antagonist.

The doses of NMI that blocked the ICV barbital place preference are similar to the doses that blocked the systemic morphine place preference. In terms of the PAG, Olmstead and Franklin (1997b) found that 5 nmol NMI administered into the PAG abolished the morphine-induced CPP. Moreover, the authors found that 2 nmol NMI administered into the PAG attenuated, but did not block, the morphine-induced place preference. The fact that 2 nmol NMI injected into the PAG blocked the ICV barbital-induced CPP is likely because the barbital CPP (difference between drug- and vehicle-paired compartments is approximately 180 seconds; Figure 21) is less robust than the morphine CPP receptor (difference is approximately 250 seconds, estimated from Figure 6 in Olmstead and Franklin, 1997b).

Administration of NMI into the posterior VTA also blocked the ICV barbital-induced CPP. Again, this antagonism occurred at the same dose of intra-VTA NMI (2 nmol) that blocked a systemic-induced morphine place preference (Olmstead and Franklin, 1997b). This is consistent with the only previous pharmacological study on barbiturate reinforcement. Systemic administration of pentobarbital (10 mg/kg) induced reward-

related shifts in ICSS thresholds in rats with electrodes implanted in the VTA (Seeger et al., 1981). Moreover, concurrent administration of naloxone (2 mg/kg) blocked the pentobarbital-induced decrease in threshold, but did not produce any effects on ICSS thresholds when administered by itself. Taken together, these findings support the idea that antagonism of μ-opioid receptors located on GABA interneurons would decrease VTA DA cell firing and block reinforcement (see Section 7.2 for discussion).

With respect to VTA opioid receptor antagonism of GABAergic drugs, Hyytia and Kiianmaa (2001) reported that ICV, but not intra-VTA, administration of CTOP and natrindole, μ- and δ-opioid receptor antagonists, respectively, suppressed oral ethanol self-administration. However, most of their cannula tips were located in the anterior VTA (i.e. 5.3 mm posterior to bregma), while $GABA_A$ agonists and ethanol are self-administered at more posterior sites (e.g. 5.3 to 6.3 mm posterior to bregma for ethanol (Rodd-Henricks et al., 2000) and 6.3 to 6.8 mm posterior to bregma for muscimol (Ikemoto et al., 1998).

The possibility that NMI may be producing its antagonist effects on a neurochemical system other than the opioid system needs to be considered. Svensson and associates (2000) examined the effect of naloxone hydrochloride on ($^{36}Cl^-$) of $GABA_A$/benzodiazepine receptor complexes in hippocampal synaptoneurosomes (HS) *in vitro*. In a concentration-dependent manner, naloxone (0.1-1000 μM) reduced 10 μM GABA-induced $^{36}Cl^-$ uptake in HS, with a 61% reduction at 1000 μM naloxone. Moreover, the co-presence of amobarbital (10 - 1000 μM) reversed the antagonistic effect of naloxone (1000 μM). This finding is consistent with reports that naloxone displaces

[^{3}H]GABA binding in homogenates of human cerebellum and in rat forebrain and cerebellum (Dingledine et al., 1978). However, high μM concentrations of naloxone were used in these studies, and the specificity of naloxone as an opioid receptor antagonist decreases as the dose of naloxone increases (Sawynok et al., 1979).

Other studies report that opioid antagonists and barbiturates may oppose each other at doses that do not produce nonspecific effects on other neurotransmitter systems (Gilbert and Martin, 1977; Gewiss et al., 1994). For example, Soderpalm and Svensson (1999) reported that depletion of brain 5-HT with 5,7-DHT pretreatment produced a significant disinhibitory effect in a modified version of Vogel's conflict test. This disinhibition was dose-dependently antagonized by systemic administration of naloxone at doses that did not affect the behaviour of sham-operated rats (e.g. 0.1 - 5.0 mg/kg). Additionally, the counteractive effect of 0.5 mg/kg naloxone on the 5,7-DHT-induced disinhibitory effect was reversed by 2 mg/kg amobarbital, and administration of amobarbital did not affect behaviour of the vehicle-treated 5,7-DHT rats. The doses of naloxone used in the study by Soderpalm and Svensson are comparable to the doses of naloxone that were found to block the pentobarbital CPP (0.02 - 2.0 mg/kg; see Chapter 3). Based on these findings, it could be argued that in the present experiment, antagonism of the ICV barbital CPP by intra-PAG or intra-posterior VTA administration of NMI may be the result of $GABA_A$ receptor blockade. As previously mentioned, the highest dose of naloxone (5 nmol) induced flight and escape behaviours in two rats that resembles intra-PAG $GABA_A$ receptor antagonism (behavioural observations, see below). However, the same argument could be applied to the evidence that morphine is antagonized by naloxone

administration into the PAG. This issue cannot be clearly resolved at this time.

6.3.2. SR 95531

The results indicate that, like NMI, administration of the higher doses of SR 95531 into either the PAG or the posterior VTA blocked the reinforcing effects of barbital in the place preference task. The finding that administration of SR 95531 into the posterior VTA blocks ICV barbital CPP supports the idea that $GABA_A$ receptor antagonism in the posterior VTA would decrease DA release by activating tonic GABAergic input (via GABA interneurons) to DA cells. Moreover, this finding is supported by a previous report that found that rats self-administered 40% fewer infusions in the posterior VTA when receiving an equimolar mixture of picrotoxin and muscimol than when receiving muscimol alone (Ikemoto et al., 1998). When administered by itself, 5 ng SR 95531 into the posterior VTA did not produce a place preference or place aversion, indicating that $GABA_A$ receptor antagonism is probably pharmacological, not behavioural.

The higher doses of SR 95531 administered into the PAG also blocked the ICV barbital-induced CPP. This is consistent with reports that microinjections of positive modulators of the $GABA_A$ receptor into the PAG raise the current threshold that induces escape behaviour produced by electrical stimulation of the PAG, while local pretreatment with the negative modulators of the $GABA_A$ receptor block this anti-aversive effect (Audi and Graeff, 1984).

Microinjections of $GABA_A$ receptor antagonists into the PAG are reported to induce defensive behaviours indicative of fear and anxiety (Di Scala et al., 1984). Indeed,

two of the animals that were injected with the highest dose of SR 95531 into the PAG displayed flight behaviour. This raised the question as to whether SR 95531 blocked the barbital CPP because its effects were aversive. While the effect of 5 ng SR 95531 administration into the PAG was not tested by itself, administration of a lower dose of SR 95531 (2.5 ng) into the PAG also blocked the barbital CPP, and did not produce a place aversion when barbital vehicle was administered into the ventricles. Therefore, like administration of SR 95531 into the posterior VTA and administration of NMI into either site, the blocking effect of intra-PAG SR 95531 on the barbital place preference was not because of the putative aversive effects of $GABA_A$ receptor antagonist.

Only one other study has examined the effects of SR 95531 on drug reinforcement. Hyytia and Koob (1995) reported that higher doses of SR 95531 (16 ng) administered into the bed nucleus of the stria terminalis and into the NAc shell suppressed responding for both ethanol and water, indicating that this effect was not selective for ethanol. In contrast, administration of lower doses (2 and 4 ng) of SR 95531 into the central nucleus of the amygdala selectively decreased responding for oral ethanol. Therefore, the doses of SR 95531 administered into the PAG or posterior VTA that blocked the ICV-induced barbital CPP are comparable to those doses administered into the central amygdala that suppress ethanol self-administration.

As mentioned in the introduction section of this chapter, recent reports indicate that SR 95531 is a more selective and specific $GABA_A$ receptor antagonist than bicuculline or its quaternary derivatives. Yu and Ho (1990) reported that SR 95531 (1 μM) significantly inhibited 500 μM pentobarbital-stimulated synaptoneurosomal chloride

uptake. Moreover, the degree of inhibition by SR 95531 was higher than that for bicuculline methiodide (54.84 ± 3.26% and 38.88 ± 2.73%, respectively), which indicates that SR 95531 could be a more potent inhibitor of barbiturate-induced chloride uptake than bicuculline. Furthermore, bicuculline and its quaternary derivatives appear to act on more than one target in the CNS. Seutin and colleagues (1997) reported that while bicuculline salts (bicuculline methiodide and methobromide), SR 95531, and picrotoxin antagonized the reduction in input resistance induced by muscimol (3 μM) in DA neurons, the bicuculline salts (1 - 300 μM) also blocked the afterhyperpolarization (AHP) of the neurons. The authors suggest that this finding may be the result of a blockade of Ca^{2+}-activated potassium channels, because the bicuculline salts did not inhibit Ca^{2+} entry, and because previous studies demonstrated that agents that possess at least one quaternary ammonium are effective Ca^{2+}-activated potassium channel blockers (Castle et al., 1993). Furthermore, the concentration-response curves for the potency of the bicuculline methobromide in antagonizing $GABA_A$ receptor responses is similar to that which produces AHP.

In summary, it appears that both opioid and $GABA_A$ receptors within the PAG and posterior VTA are involved in the central reinforcing effects of barbital. The implications of these findings are discussed in the next section (Chapter 7), and a model based on the neuropharmacology of barbiturate reinforcement is put forward.

CHAPTER 7:

GENERAL DISCUSSION

7.1. Evaluation of Barbiturate Reinforcement in the CPP Paradigm

The findings from the experiments presented in this dissertation demonstrate that two different barbiturates are reinforcing in rats in the place preference paradigm. These findings are consistent with animal and human self-administration experiments (Griffiths et al., 1979; Ator and Griffiths, 1987) and human choice preference tests (Griffiths et al., 1980), but are in contrast to previous studies that reported that barbiturates are aversive, rather than reinforcing, in the CPP task (Mucha and Iversen, 1984; Wilks and File, 1988; Lew and Parker, 1998). As discussed, the discrepant results may be due to differences in methodological and procedural parameters. Furthermore, it should be noted that both conditioned place preferences and conditioned place aversions have also been reported with other drugs. Some researchers report that apomorphine (Spyraki et al., 1982b; Parker, 1992) and phencyclidine (Marglin et al., 1989) produce a place preference, while others report that they produce place aversions (Best et al., 1973; Iwamoto, 1985a; Miyamoto et al., 2000). Place preferences and place aversions have also been reported for nicotine (Acquas et al., 1989; Jorenby et al., 1990; Risinger and Oakes, 1995). Interestingly, nicotine and ethanol are reported to only produce place preferences (and not aversions) when centrally administered (Iwamoto, 1990; Walker and Ettenberg, 2001; Walker and Ettenberg, 2002). This suggests that peripheral mechanisms may contribute to the place aversion obtained with some drugs of abuse. Moreover, a recent study reported that some rats that did not learn to self-administer cocaine displayed a conditioned place aversion to cocaine (Rademacher et al., 2000). Although only a few

rats were classified as non-self-administrators and showed the subsequent place aversion, the authors suggest that this finding demonstrates that the effects of a reinforcing drug, such as cocaine, are aversive to some animals.

The place paradigm is based on the assumption that animals associate the drug effect with stimuli in a particular compartment, and must remember this association in order to display a preference on test day. White and Carr (1985) found that rats did not show a preference for a compartment paired with saccharin, but did for one that was paired with sucrose. Since the same amounts of both compounds were consumed, they were both considered to be rewarding. However, the saccharin CPP was only observed when post-training injections of glucose or amphetamine were used to improve memory for the pairings. Given this, it could be argued that, in addition to their reinforcing effects, drugs that improve memory processes would be more likely to induce a place preference and, conversely, drugs that impair memory processes would be less likely to induce a CPP. Indeed, barbiturates are reported to disrupt certain memory processes. For example, Tomaz and colleagues (1982) reported that administration of barbital impairs retention, but not acquisition, of an appetitive task using a Y-maze. Moreover, like morphine and ethanol, barbiturates are reported to produce state-dependent learning (SDL) (Overton, 1966; Hill et al., 1971). SDL refers to the fact that a response that has been learned or acquired while the animal is in a certain state can only be retrieved when the animal is in a similar, but not different, state. Indeed, it was found here that the highest doses of pentobarbital (Chapter 2) and barbital (Chapter 4) did not produce a CPP. It is possible that at these doses, barbiturates produce SDL. However, the doses of pentobarbital and

barbital that produced a significant place preference were tested in a drug-free state. This suggests that the barbiturate-induced CPP is not prevented by SDL. Moreover, other drugs that are reported to disrupt memory processes, such as the NMDA receptor antagonist MK-801 (Mondadori et al., 1989; Heale and Harley, 1990) also induce a conditioned place preference (Hoffman, 1994; Panos et al., 1999).

Alternatively, the fact that memory-suppressing drugs would impair habituation suggests that such drugs may be *more* likely to induce a place preference. This interpretation, also based on SDL learning, proposes that animals are less familiar with the drug-paired compartment on test day (since they experienced that compartment in a drugged state during conditioning), and show a preference to that compartment mainly because it is novel. Indeed, Bardo and associates (1989) reported that rats significantly preferred a novel compartment (e.g. no previous exposure) over a compartment that they were familiar with (e.g. previously exposed to for eight 30 minute sessions). Carr and colleagues (1988) found that complete novelty results in an initial period of avoidance of the novel side (e.g. during the first 5 minutes of a 20 minute test), but animals prefer a partially novel compartment over the entire 20 minute test period. These findings suggest that if conditioning with a drug prevents familiarization of a compartment, then the animal may prefer the compartment because it is novel, not reinforcing. Given this, it could be argued that the animals display a place preference to barbiturates because of their memory-impairing, not reinforcing, effects. However, this argument is not strongly supported by the barbiturate CPP findings. Firstly, it is reasonable to assume that the higher the dose of barbiturate, the more the memory-impairing effect. Thus, the

magnitude of the barbiturate CPP should increase as the dose of barbiturate increases. This pattern of predicted results was not found in the present experiments. Here, the barbiturate place preferences all occurred within a narrow dose range, with low and high doses being ineffective and intermediate doses being reinforcing. Secondly, in the experiment by Bardo and associates (1998), while animals spent significantly more time in the novel compartment, rats displayed more horizontal (line-crossing) and vertical (rearing) activity in the familiar compartment. The authors suggest that despite the preference for the novel compartment, approach responses to novelty may be counteracted by an increase in freezing or grooming responses to novelty. In contrast, in the present experiments, across all of the barbiturate CPPs, the animals that spent significantly more time in the drug-paired compartments also displayed more activity in those compartments. This elevation of locomotor activity only with effective doses of barbiturates suggests that the increase in activity is a response to the conditioned effects of the drug (e.g. drug-seeking behaviour), not a response to novelty. Finally, findings from other studies indicate that drug-induced preferences are not because of perceived novelty of the compartments. Parker (1992) found that using a 3 or 4-compartment apparatus, rats demonstrated a place preference to either amphetamine-, morphine-, or apomorphine-paired compartments compared to saline-paired, partially novel, or completely novel compartments. Taken together, the findings indicate that the place preferences induced by barbiturates are due to their reinforcing, not memory-impairing, effects.

6.2. Neuropharmacology of Barbiturate Reinforcement

As found in Chapter 5, the barbital place preference appears to be mediated by the PAG and the posterior VTA, and these reinforcing effects involve both the opioid and GABAergic system (Chapter 6). Morphine reinforcement, assessed in the self-administration and CPP paradigm, is also mediated by the PAG and VTA (van der Kooy et al., 1982; Cazala, 1990; David and Cazala, 1994b; Olmstead and Franklin, 1997b). However, evidence from anatomical and behavioural studies suggest that these areas do not share a common function. It is, therefore, possible that intra-PAG and intra-posterior VTA barbital injections produce a CPP through different mechanisms.

While the VTA is part of the neural system that mediates reward and approach behaviour (Wise and Bozarth, 1987), the PAG is part of the neural system that mediates defensive and escape behaviours (Carrive, 1993; Behbehani, 1995). Microinjections of pentobarbital into the PAG increase the aversive threshold of PAG stimulation (the lowest electrical current intensity that induces flight behaviour), while administration of picrotoxin and bicuculline into the PAG induces flight behaviour similar to that produced by PAG electrical stimulation (Graeff et al., 1986b). This type of defensive behaviour is elicited by stimulation of the rostral dorsolateral PAG (Morgan et al., 1998), where the greatest density of GABA-immunoreactive neurons are found (Lovick and Paul, 1999). In contrast, stimulation of the caudal ventrolateral PAG induces immobility (Morgan et al., 1998). Furthermore, this defensive or flight behaviour is specific to the $GABA_A$ receptor, since $GABA_B$ receptor agonists are ineffective (Graeff et al., 1986ba). It was found in

Chapter 5 that cannula aimed at the rostral dorsolateral portion of the PAG induced a place preference, while areas dorsal or lateral to this area did not. Given this, it was suggested in Chapters 5 and 6 that administration of barbital into the PAG produces a place preference because it inhibits aversive or anxiogenic aspects of CPP conditioning. However, while the PH receives projections from the PAG (Abrahamson and Moore, 2001) and also mediates defensive/escape behaviours, injections of barbital into the PH did not induce a CPP (Chapter 5). Furthermore, there is a strong projection from the amygdala to the PAG (Paxinos, 1995) and it has been suggested that threatening stimuli activate a network in the amygdala that projects to the PAG which, in turn, leads to defense reactions, analgesia, and autonomic responses (Behbehani, 1995). However, like the PH, injections into the lateral or central nucleus of the amygdala also did not induce a barbital place preference (Chapter 5). Given the major connections between the PH, amygdala, and PAG, it would be reasonable to assume that if the intra-PAG barbital CPP was produced by the anxiolytic effects of barbital, then similar place preferences would also be obtained from the PH and amygdala. Therefore, it is not likely that intra-PAG barbital administration produces a place preference because it inhibits anxiogenic aspects of CPP conditioning.

The experiments in Chapter 6 demonstrated that the ICV barbital place preference is blocked by administration of opioid (NMI) and $GABA_A$ receptor (SR 95531) antagonists into the PAG. This suggests that blockade of either of these systems in the PAG is sufficient to disrupt the reinforcing effects of barbital. The precise mechanism of this blockade is not known, and there is little evidence that links the PAG to the VTA.

Ascending projections from the PAG to the NAc (Paxinos, 1995) and descending projections from the PAG to the pedunculopontine tegmental nucleus which, in turn, projects to the substantia nigra and areas of the basal ganglia (Jackson and Crossman, 1983) may be involved in the intra-PAG reinforcing effects of barbital. However, the anatomical basis of this reinforcing effect remains to be determined.

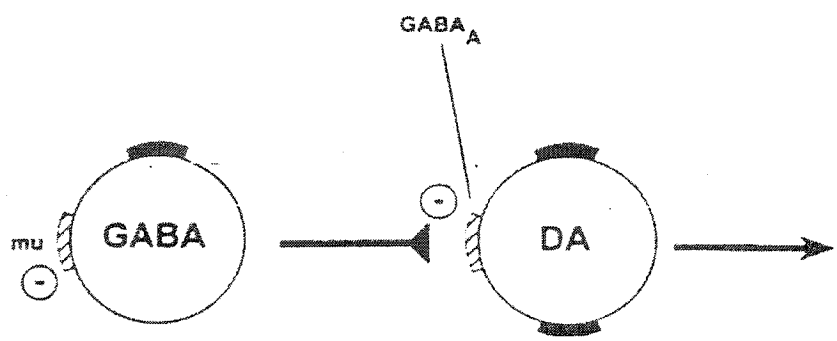
In Chapter 5, it was found that administration of barbital into the posterior VTA induced a place preference. Therefore, it appears that the same mechanism that mediates the reinforcing effects of $GABA_A$ receptor agonists and ethanol in the posterior VTA also mediates barbiturate reinforcement in the CPP paradigm. As shown in Figure 27, in the anterior VTA, activation of tonic $GABA_A$ mediated inhibition of DA neurons would result in decreased DA cell firing, while antagonism of $GABA_A$ receptors would activate DA neurons and increase cell firing. In contrast, in the posterior VTA, $GABA_A$ receptors are located on GABA interneurons, while $GABA_B$ receptors mediate tonic inhibition of DA neurons. Here, activation of $GABA_A$ receptors would inhibit the firing of GABA interneurons, resulting in disinhibition of DA neurons. Additionally, this model predicts that antagonism of $GABA_A$ receptors in the posterior VTA would decrease DA cell firing. Indeed, it was found here that administration of SR 95531 into the posterior VTA blocked the centrally-induced reinforcing effects of barbital (Chapter 6).

In terms of opioid-mediated reinforcement, activation of μ-opioid receptors on GABA interneurons in both regions of the VTA would disinhibit VTA DA neurons and promote reinforcement. In contrast, antagonism of μ-opioid receptors would decrease VTA DA cell firing and block reinforcement. In the experiment in Chapter 6,

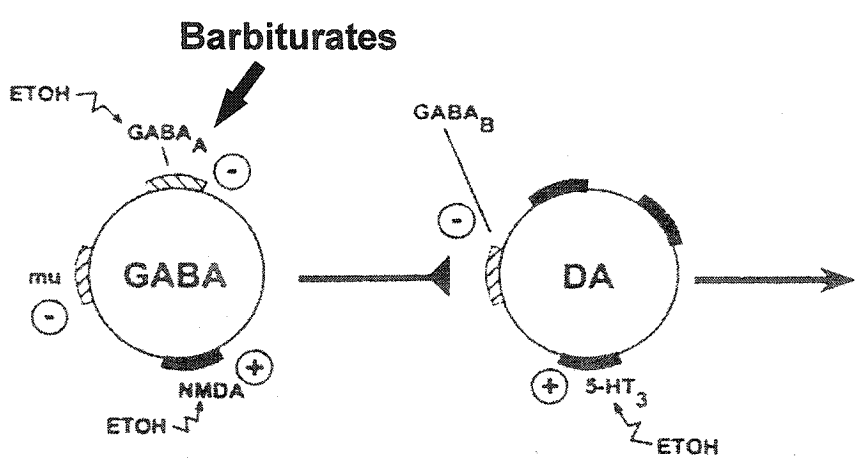
Figure 27. Hypothetical simplified circuits for GABAergic reinforcement in the VTA. Activation of mu-opioid receptors in both the anterior and posterior VTA results in disinhibition of DA neurons. In the anterior VTA, blockade of $GABA_A$ receptors on DA neurons reduces tonic inhibition of the DA neurons. This disinhibition results in increased firing rates of VTA DA neurons and contributes to reinforcement processes. In the posterior VTA, tonic inhibition of DA neuorns is mediated by $GABA_B$ receptors. Here, $GABA_A$ receptors are located on GABA interneurons and activation of $GABA_A$ receptors results in disinhibition of DA neurons and produces reinforcement. In addition to its effects on $GABA_A$ receptors in the posterior VTA, ethanol (ETOH) may activate 5-HT3 receptors located on DA neurons and/or inhibit NMDA receptors that are present on GABA interneurons. Modified from McBride and associates (1999).

Hypothetical Simplified Circuits For GABAergic Reinforcement in the VTA

Anterior



Posterior



administration of NMI into the posterior VTA was found to block barbital reinforcement. While not tested, it is likely that NMI injections into the anterior VTA would also block barbital reinforcement. However, higher doses of NMI in the anterior VTA may be required to block the barbital-induced CPP, since the density of opioid receptors is greater within the posterior VTA (Mansour et al., 1995).

The dopaminergic system is also involved in barbiturate reinforcement, since systemic administration of the DA D_2 receptor antagonist, eticlopride, blocked the pentobarbital CPP (Chapter 3). Given that the reinforcing effects of barbital are ultimately related to increased transmission in the mesolimbic DA system, it is reasonable to assume that intracranial administration of DA receptor antagonists would also block barbiturate reinforcement. Administration of DA D_2 receptor antagonists into the VTA would not be expected to block barbiturate reinforcement, since DA receptor antagonists would increase VTA DA cell firing by blocking presynaptic DA receptors (Roth, 1984). However, given that intra-NAc administration of the DA receptor antagonist, fluphenazine, attenuates the development of a CPP produced by ICV ethanol administration (Walker and Ettenberg, 2002), it is presumed that injections of DA receptor antagonists into the NAc would also block a barbiturate-induced CPP.

6.3. Concluding Remarks

In summary, the experiments presented in this dissertation are the first to establish the neuropharmacological mechanisms involved in barbiturate reinforcement. In Chapter 2, it was demonstrated that, like other drugs of abuse, pentobarbital produces a significant place preference in rats. In Chapter 3, the pentobarbital CPP was found to be mediated by GABAergic, dopaminergic, and opioid systems, since pretreatment with the respective antagonists bicuculline, eticlopride, and naloxone blocked the pentobarbital place preference. Systemic administration of the longer-acting barbiturate, barbital, also induced a place preference, and this reinforcing effect was determined to be centrally mediated (Chapter 4). Moreover, findings from Chapters 5 and 6 demonstrated that the PAG and posterior VTA mediate the reinforcing effects of barbital in the CPP paradigm and both opioid and GABAergic systems are involved in the central reinforcing effects of barbital.

In conclusion, the findings presented in this dissertation assert that barbiturates are *not* anomalous compared to other drugs in terms of the mechanisms by which they produce reinforcement. Instead, the present findings suggest that barbiturate reinforcement is in fact mediated by the same neural substrates and neurochemical systems as other drugs of abuse, such as opiates and ethanol.

References

1. Abrahamson E.E., Moore R.Y. (2001) The posterior hypothalamic area: chemoarchitecture and afferent connections. *Brain Research 889*: 1-22

2. Acquas E., Carboni E., Leone P., Di Chiara G. (1989) SCH 23390 blocks drug-conditioned place-preference and place-aversion: anhedonia (lack of reward) or apathy (lack of motivation) after dopamine-receptor blockade? *Psychopharmacology 99*: 151-155

3. Agmo A., Federman I., Navarro V., Padua M., Velazquez G. (1993) Reward and reinforcement produced by drinking water: role of opioids and dopamine receptor subtypes. *Pharmacology Biochemistry and Behavior 46*: 183-194

4. Agmo A., Galvan A., Talamantes B. (1995) Reward and reinforcement produced by drinking sucrose: two processes that may depend on different neurotransmitters. *Pharmacology Biochemistry and Behavior 52*: 403-414

5. Akwa Y., Purdy R.H., Koob G.F., Britton K.T. (1999) The amygdala mediates the anxiolytic-like effect of the neurosteroid allopregnanolone in rat. *Behavioural Brain Research 106*: 119-125

6. Allgulander C. (1986) History and current status of sedative-hypnotic drug use and abuse. *Acta Psychiatrica Scandinavica 73*: 465-478

7. AMA Committee on Alcoholism and Addiction (1965) Dependence on barbiturates and other sedative drugs. *Journal of the American Medical Association 193*: 673-677

8. Amalric M., Cline E.J., Martinez J.L. Jr., Bloom F.E., Koob G.F. (1987) Rewarding properties of beta-endorphin as measured by conditioned place preference. *Psychopharmacology 91*: 14-19

9. Anseloni V.C., Coimbra N.C., Morato S., Brandao M.L. (1999) A comparative study of the effects of morphine in the dorsal periaqueductal gray and nucleus accumbens of rats submitted to the elevated plus-maze test. *Experimental Brain Research 129*: 260-268

10. Arnt J., Sanchez C., Lenz S.M., Madsen U., Krogsgaard-Larsen P. (1995) Differentiation of in vivo effects of AMPA and NMDA receptor ligands using drug discrimination methods and convulsant/anticonvulsant activity. *European Journal of Pharmacology 285*: 289-297

11. Arnt J., Scheel-Kruger J. (1979) GABA in the ventral tegmental area: differential regional effects on locomotion, aggression and food intake after microinjection of GABA agonists and antagonists. *Life Sciences 25*: 1351-1360

12. Asin K.E., Wirtshafter D., Tabakoff B. (1985) Failure to establish a conditioned place preference with ethanol in rats. *Pharmacology Biochemistry and Behavior 22*: 169-173

13. Ator N.A., Griffiths R.R. (1987) Self-administration of barbiturates and benzodiazepines: a review. *Pharmacology Biochemistry and Behavior 27*: 391-398

14. Audi E.A., Graeff F.G. (1984) Benzodiazepine receptors in the periaqueductal grey mediate anti-aversive drug action. *European Journal of Pharmacology 103*: 279-285

15. Bailey D.N., Jatlow P.I. (1975) Barbital overdose and abuse. *American Journal of Clinical Pathology 64*: 291-296

16. Bals-Kubik R., Ableitner A., Herz A., Shippenberg T.S. (1993) Neuroanatomical sites mediating the motivational effects of opioids as mapped by the conditioned place preference paradigm in rats. *Journal of Pharmacology and Experimental Therapeutics 264*: 489-495

17. Bardo M.T. (1998) Neuropharmacological mechanisms of drug reward: beyond dopamine in the nucleus accumbens. *Critical Reviews in Neurobiology 12*: 37-67

18. Bardo M.T., Bevins R.A. (2000) Conditioned place preference: what does it add to our preclinical understanding of drug reward? *Psychopharmacology 153*: 31-43

19. Bardo M.T., Neisewander J.L., Pierce R.C. (1989) Novelty-induced place preference behavior in rats: effects of opiate and dopaminergic drugs. *Pharmacology Biochemistry and Behavior 32*: 683-689

20. Bardo M.T., Valone J.M., Bevins R.A. (1999) Locomotion and conditioned place preference produced by acute intravenous amphetamine: role of dopamine receptors and individual differences in amphetamine self-administration. *Psychopharmacology 143*: 39-46

21. Barrett J.E., Witkin J.M. (1976) Interaction of d-amphetamine with pentobarbital and chlordiazepoxide: effects on punished and unpunished behavior of pigeons. *Pharmacology Biochemistry and Behavior 5*: 285-292

22. Barry H. (1974) Classification of drugs according to their discriminable effects in rats. *Federation Proceedings 33*: 1814-1824

23. Basbaum, A.I., Fields, H.L. (1978) Endogenous pain control mechanisms: review and hypothesis. *Annals of Neurology 4*: 451-462

24. Beach H.D. (1957) Morphine addiction in rats. *Canadian Journal of Psychology 11*: 104-112

25. Bechara A., van der Kooy D. (1985) Opposite motivational effects of endogenous opioids in brain and periphery. *Nature 314*: 533-534

26. Bedingfield J.B., King D.A., Holloway F.A. (1998) Cocaine and caffeine - conditioned place preference, locomotor activity, and additivitiy. *Pharmacology Biochemistry and Behavior 61*: 291-296

27. Behbehani M.M. (1995) Functional characteristics of the midbrain periaqueductal gray. *Progress in Neurobiology 46*: 575-605

28. Best P.J., Best M.R., Mickley G.A. (1973) Conditioned aversion to distinct environmental stimuli resulting from gastrointestinal distress. *Journal of Comparative and Physiological Psychology 85*: 250-257

29. Biala G., Langwinski R. (1996) Rewarding properties of some drugs studied by place preference conditioning. *Polish Journal of Pharmacology 48*: 425-430

30. Bienkowski P., Kostowski W., Koros E. (1999) Ethanol-reinforced behaviour in the rat: effects of naltrexone. *European Journal of Pharmacology 374*: 321-327

31. Bienkowski P., Kuca P., Kostowski W. (1995) Conditioned place preference after prolonged pre-exposure to ethanol. *Polish Journal of Pharmacology 47*: 189-191

32. Bigelow G.E., Griffiths R.R., Liebson I.A. (1976) Effects of response requirement upon human sedative self-administration and drug-seeking behavior. *Pharmacology Biochemistry and Behavior 5*: 681-685

33. Bird M., Kornetsky C. (1990) Dissociation of the attentional and motivational effects of pimozide on the threshold for rewarding brain stimulation. *Neuropsychopharmacology 3*: 33-40

34. Boisse N.R., Okamoto M. (1978) Physical dependence to barbital compared to pentobarbital. I. "Chronically equivalent" dosing method. *Journal of Pharmacology*

and Experimental Therapeutics 204: 497-506

35. Borisenko S.A., Meng Q.H., Rauhala P., Mannisto P.T. (1996) Neurochemical mediators of anxiety have inconsistent effects on hypothalamic self-stimulation in rats. *Pharmacology and Toxicology 78*: 354-360

36. Bozarth M.A. (1987a) Conditioned place preference: A parametric analysis using systemic heroin injections. In: Bozarth MA (ed) *Methods of assessing the reinforcing properties of abused drugs*. Springer-Verlag, New York, pp 241-273

37. Bozarth M.A. (1987b) Neuroanatomical boundaries of the reward-relevant opiate-receptor field in the ventral tegmental area as mapped by the conditioned place preference method in rats. *Brain Research 414*: 77-84

38. Bozarth M.A. (1990) Evidence for the rewarding effects of ethanol using the conditioned place preference method. *Pharmacology Biochemistry and Behavior 35*: 485-487

39. Bozarth M.A., Wise R.A. (1981) Intracranial self-administration of morphine into the ventral tegmental area in rats. *Life Sciences 28*: 551-555

40. Brady J.V., Griffiths R.R. (1976) Behavioral procedures for evaluating the relative abuse potential of CNS drugs in primates. *Federation Proceedings 35*: 2245-2253

41. Brebner K., Phelan R., Roberts D.C. (2000) Intra-VTA baclofen attenuates cocaine self-administration on a progressive ratio schedule of reinforcement. *Pharmacology*

Biochemistry and Behavior 66: 857-862

42. Brodie M.S., Appel S.B. (1998) The effects of ethanol on dopaminergic neurons of the ventral tegmental area studied with intracellular recording in brain slices. *Alcoholism: Clinical and Experimental Research 22*: 236-244

43. Brodie M.S., Shefner S.A., Dunwiddie T.V. (1990) Ethanol increases the firing rate of dopamine neurons of the rat ventral tegmental area in vitro. *Brain Research 508*: 65-69

44. Brodie M.S., Trifunovic R.D., Shefner S.A. (1995) Serotonin potentiates ethanol-induced excitation of ventral tegmental area neurons in brain slices from three different rat strains. *Journal of Pharmacology and Experimental Therapeutics 273*: 1139-1146

45. Broekkamp C.L., Phillips A.G. (1979) Facilitation of self-stimulation behavior following intracerebral microinjections of opioids into the ventral tegmental area. *Pharmacology Biochemistry and Behavior 11*: 289-295

46. Brown E.E., Fibiger H.C. (1993) Differential effects of excitotoxic lesions of the amygdala on cocaine-induced conditioned locomotion and conditioned place preference. *Psychopharmacology 113*: 123-130

47. Budavari S., O'Neil M.J. (1996) *The Merck Index: An encyclopedia of chemicals, drugs, and biologicals*, 12 edn. Merck & Co. Inc., Rahway, NJ

48. Buggy D.J., Nicol B., Rowbotham D.J., Lambert D.G. (2000) Effects of intravenous anesthetic agents on glutamate release - A role for GABA(A) receptor-mediated inhibition. *Anesthesiology 92*: 1067-1073

49. Caine S.B., Koob G.F. (1994) Effects of dopamine D-1 and D-2 antagonists on cocaine self-administration under different schedules of reinforcement in the rat. *Journal of Pharmacology and Experimental Therapeutics 270*: 209-218

50. Campbell A.D., Kohl R.R., McBride W.J. (1996) Serotonin-3 receptor and ethanol-stimulated somatodendritic dopamine release. *Alcohol 13*: 569-574

51. Carden S.E., Coons E.E. (1990) Diazepam's impact on self-stimulation but not stimulation-escape suggests hedonic modulation. *Behavioral Neuroscience 104*: 56-61

52. Carlson R.H., Lydic R. (1976) The effects of ethanol upon threshold and response rate for self-stimulation. *Psychopharmacology 50*: 61-64

53. Carney J.M., Landrum R.W., Cheng M.S., Seale T.W. (1991) Establishment of chronic intravenous drug self-administration in the C57BL/6J mouse. *Neuroreport 2*: 477-480

54. Carr G.D., Fibiger H.C., Phillips A.G. (1989) Conditioned place preference as a measure of drug reward. In: Liebman JM, Cooper SJ (eds) *The neuropharmacological basis of reward.* Oxford University Press, Oxford, pp 264-

55. Carr G.D., Phillips A.G., Fibiger H.C. (1988) Independence of amphetamine reward from locomotor stimulation demonstrated by conditioned place preference. *Psychopharmacology 94*: 221-226

56. Carr G.D., White N.M. (1983) Conditioned place preference from intra-accumbens but not intra-caudate amphetamine injections. *Life Sciences 33*: 2551-2557

57. Carr G.D., White N.M. (1986) Anatomical disassociation of amphetamine's rewarding and aversive effects: an intracranial microinjection study. *Psychopharmacology 89*: 340-346

58. Carrive P. (1993) The periaqueductal gray and defensive behavior: functional representation and neuronal organization. *Behavioural Brain Research 58*: 27-47

59. Castle N.A., Haylett D.G., Morgan J.M., Jenkinson D.H. (1993) Dequalinium: a potent inhibitor of apamin-sensitive K+ channels in hepatocytes and of nicotinic responses in skeletal muscle. *European Journal of Pharmacology 236*: 201-207

60. Cazala P. (1990) Dose-dependent effects of morphine differentiate self-administration elicited from lateral hypothalamus and mesencephalic central gray area in mice. *Brain Research 527*: 280-285

61. Cazala P., Darracq C., Saint-Marc M. (1987) Self-administration of morphine into the lateral hypothalamus in the mouse. *Brain Research 416*: 283-288

62. Cervo L., Samanin R. (1995) Effects of dopaminergic and glutamatergic receptor antagonists on the acquisition and expression of cocaine conditioning place preference. *Brain Research 673*: 242-250

63. Cherubini E., Gaiarsa J.L., Ben Ari Y. (1991) GABA: an excitatory transmitter in early postnatal life. *Trends in Neurosciences 14*: 515-519

64. Chester J.A., Cunningham C.L. (1999) GABA(A) receptors modulate ethanol-induced conditioned place preference and taste aversion in mice. *Psychopharmacology 144*: 363-372

65. Chester J.A., Cunningham C.L. (2002) GABA(A) receptor modulation of the rewarding and aversive effects of ethanol. *Alcohol 26*: 131-143

66. Chevrette J., Stellar J.R., Hesse G.W., Markou A. (2002) Both the shell of the nucleus accumbens and the central nucleus of the amygdala support amphetamine self-administration in rats. *Pharmacology Biochemistry and Behavior 71*: 501-507

67. Churchill L., Dilts R.P., Kalivas P.W. (1992) Autoradiographic localization of gamma-aminobutyric acidA receptors within the ventral tegmental area. *Neurochemical Research 17*: 101-106

68. Ciccocioppo R., Panocka I., Froldi R., Quitadamo E., Massi M. (1999) Ethanol induces conditioned place preference in genetically selected alcohol-preferring rats. *Psychopharmacology 141*: 235-241

69. Collins R.J., Weeks J.R., Cooper M.M., Good P.I., Russell R.R. (1984) Prediction of abuse liability of drugs using IV self-administration by rats. *Psychopharmacology 82*: 6-13

70. Cooper B.R., Breese G.R. (1975) A role for dopamine in the psychopharmacology of electrical self-stimulation. *NIDA Research Monograph 3*: 63-70

71. Cooper J.R., Bloom F.E., Roth R.H. (1996) Amino acid transmitters. In: House J (ed) *The biochemical basis of neuropharmacology*. Oxford University Press, New York pp 126-193

72. Cooper S.J., Joyce D., Summerfield A. (1969) Self-stimulation of the brain after administration of an amphetamine-barbiturate mixture. British Journal of Pharmacology 36: 192-193

73. Corbett D., Wise R.A. (1980) Intracranial self-stimulation in relation to the ascending dopaminergic systems of the midbrain: a moveable electrode mapping study. *Brain Research 185*: 1-15

74. Corrigall W.A., Linseman M.A. (1988) Conditioned place preference produced by intra-hippocampal morphine. *Pharmacology Biochemistry and Behavior 30*: 787-789

75. Corrigall W.A., Vaccarino F.J. (1988) Antagonist treatment in nucleus accumbens or periaqueductal grey affects heroin self-administration. *Pharmacology Biochemistry*

and Behavior 30: 443-450

76. Costa E. (1998) From GABAA receptor diversity emerges a unified vision of GABAergic inhibition. *Annual Review of Pharmacology and Toxicology 38*: 321-350

77. Crow T.J. (1973) Catecholamine-containing neurones and electrical self-stimulation. 2. A theoretical interpretation and some psychiatric implications. *Psychological Medicine 3*: 66-73

78. Crow T.J., Arbuthnott G.W. (1972) Function of catecholamine-containing neurones in mammalian central nervous system. *Nature - New Biology 238*: 245-246

79. Cunningham C.L., Dickinson S.D., Okorn D.M. (1995) Naloxone facilitates extinction but does not affect acquisition or expression of ethanol-induced conditioned place preference. *Experimental and Clinical Psychopharmacology 3*: 330-343

80. Cunningham C.L., Henderson C.M., Bormann N.M. (1998) Extinction of ethanol-induced conditioned place preference and conditioned place aversion: effects of naloxone. *Psychopharmacology 139*: 62-70

81. Cunningham C.L., Malott D.H., Dickinson S.D., Risinger F.O. (1992) Haloperidol does not alter expression of ethanol-induced conditioned place preference. *Behavioural Brain Research 50*: 1-5

82. Cunningham C.L., Prather L.K. (1992) Conditioning trial duration affects ethanol-induced conditioned place preference in mice. *Animal Learning and Behavior 20*: 187-194

83. Czachowski C.L., Chappell A.M., Samson H.H. (2001) Effects of raclopride in the nucleus accumbens on ethanol seeking and consumption. *Alcoholism: Clinical and Experimental Research 25*: 1431-1440

84. Dahlstrom A., Fuxe K. (1964) Evidence for the existence of monoamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiologica Scandinavica 232*: 1-55

85. Daniell L.C. (1990) The noncompetitive N-methyl-D-aspartate antagonists, MK-801, phencyclidine and ketamine, increase the potency of general anesthetics. *Pharmacology Biochemistry and Behavior 36*: 111-115

86. David V., Cazala P. (1994a) A comparative study of self-administration of morphine into the amygdala and the ventral tegmental area in mice. *Behavioural Brain Research 65*: 205-211

87. David V., Cazala P. (1994b) Differentiation of intracranial morphine self-administration behavior among five brain regions in mice. *Pharmacology Biochemistry and Behavior 48*: 625-633

88. David V., Cazala P. (2000) Anatomical and pharmacological specificity of the

rewarding effect elicited by microinjections of morphine into the nucleus accumbens of mice. *Psychopharmacology 150*: 24-34

89. David V., Durkin T.P., Cazala P. (1997) Self-administration of the GABAA antagonist bicuculline into the ventral tegmental area in mice: dependence on D2 dopaminergic mechanisms. *Psychopharmacology 130*: 85-90

90. de Olmos J.S., Heimer L. (1999) The concepts of the ventral striatopallidal system and extended amygdala. *Annals of the New York Academy of Sciences 877*: 1-32

91. de Wit H., Griffiths R.R. (1991) Testing the abuse liability of anxiolytic and hypnotic drugs in humans. *Drug and Alcohol Dependence 28*: 83-111

92. de Wit H., Pierri J., Johanson C.E. (1989) Assessing pentobarbital preference in normal volunteers using a cumulative dosing procedure. *Psychopharmacology 99*: 416-421

93. de Wit H., Uhlenhuth E.H., Johanson C.E. (1986) Individual differences in the reinforcing and subjective effects of amphetamine and diazepam. *Drug and Alcohol Dependence 16*: 341-360

94. DeNoble V.J., Mele P.C., Porter J.H. (1985) Intravenous self-administration of pentobarbital and ethanol in rats. *Pharmacology Biochemistry and Behavior 23*: 759-763

95. Deroche V., Le Moal M., Piazza P.V. (1999) Cocaine self-administration increases the incentive motivational properties of the drug in rats. *European Journal of Neuroscience 11*: 2731-2736

96. Devine D.P., Leone P., Carlezon W.A. Jr., Wise R.A. (1993) Ventral mesencephalic delta opioid receptors are involved in modulation of basal mesolimbic dopamine neurotransmission: an anatomical localization study. *Brain Research 622*: 348-352

97. Devine D.P., Wise R.A. (1994) Self-administration of morphine, DAMGO, and DPDPE into the ventral tegmental area of rats. *Journal of Neuroscience 14*: 1978-1984

98. Di Chiara G. (1995) The role of dopamine in drug abuse viewed from the perspective of its role in motivation. *Drug and Alcohol Dependence 38*: 95-137

99. Di Chiara G., Acquas E., Tanda G. (1996) Ethanol as a neurochemical surrogate of conventional reinforcers: the dopamine-opioid link. *Alcohol 13*: 13-17

100. Di Chiara G., Imperato A. (1986) Preferential stimulation of dopamine release in the nucleus accumbens by opiates, alcohol, and barbiturates: studies with transcerebral dialysis in freely moving rats. *Annals of the New York Academy of Sciences 473*: 367-381

101. Di Chiara G., Imperato A. (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats.

Proceedings of the National Academy of Sciences of the United States of America 85: 5274-5278

102. Di Scala G., Sandner G. (1989) Conditioned place aversion produced by microinjections of semicarbazide into the periaqueductal gray of the rat. *Brain Research 483*: 91-97

103. Di Scala G., Schmitt P., Karli P. (1984) Flight induced by infusion of bicuculline methiodide into periventricular structures. *Brain Research 309*: 199-208

104. Dingledine R., Iversen L.L., Breuker E. (1978) Naloxone as a GABA antagonist: evidence from iontophoretic, receptor binding and convulsant studies. *European Journal of Pharmacology 47*: 19-27

105. Dodson B.A., Urh R.R., Miller K.W. (1990) Relative potencies for barbiturate binding to the Torpedo acetylcholine receptor. *British Journal of Pharmacology 101*: 710-714

106. Edmonds D.E., Gallistel C.R. (1974) Parametric analysis of brain stimulation reward in the rat: III. Effect of performance variables on the reward summation function. *Journal of Comparative and Physiological Psychology 87*: 876-883

107. Elekes I., Patthy A., Lang T., Palkovits M. (1986) Concentrations of GABA and glycine in discrete brain nuclei. Stress-induced changes in the levels of inhibitory amino acids. *Neuropharmacology 25*: 703-709

108. Esposito R., Kornetsky C. (1977) Morphine lowering of self-stimulation thresholds: lack of tolerance with long-term administration. *Science 195*: 189-191

109. Esposito R.U., Motola A.H., Kornetsky C. (1978) Cocaine: acute effects on reinforcement thresholds for self-stimulation behavior to the medial forebrain bundle. *Pharmacology Biochemistry and Behavior 8*: 437-439

110. Ettenberg A., Pettit H.O., Bloom F.E., Koob G.F. (1982) Heroin and cocaine intravenous self-administration in rats: mediation by separate neural systems. *Psychopharmacology 78*: 204-209

111. Everitt B.J., Parkinson J.A., Olmstead M.C., Arroyo M., Robledo P., Robbins T.W. (1999) Associative processes in addiction and reward. The role of amygdala-ventral striatal subsystems. *Annals of the New York Academy of Sciences 877*: 412-438

112. Fejer D., Smart R. (1973) The use of psychoactive drugs by adults. *Canadian Psychiatric Association Journal 18*: 313-320

113. ffrench-Mullen J.M., Barker J.L., Rogawski M.A. (1993) Calcium current block by (-)-pentobarbital, phenobarbital, and CHEB but not (+)-pentobarbital in acutely isolated hippocampal CA1 neurons: comparison with effects on GABA-activated Cl- current. *Journal of Neuroscience 13*: 3211-3221

114. File S.E. (1986) Aversive and appetitive properties of anxiogenic and anxiolytic agents. *Behavioural Brain Research 21*: 189-194

115. Finlay J.M., Damsma G., Fibiger H.C. (1992) Benzodiazepine-induced decreases in extracellular concentrations of dopamine in the nucleus accumbens after acute and repeated administration. *Psychopharmacology 106*: 202-208

116. Finn D.A., Phillips T.J., Okorn D.M., Chester J.A., Cunningham C.L. (1997) Rewarding effect of the neuroactive steroid 3 alpha-hydroxy-5 alpha- pregnan-20-one in mice. *Pharmacology Biochemistry and Behavior 56*: 261-264

117. Flynn E.J., Spector S. (1972) Determination of barbiturate derivatives by radioimmunoassay. *Journal of Pharmacology and Experimental Therapeutics 181*: 547-554

118. Franklin K.B.J., Abbott F.V. (1993) Pentobarbital, diazepam, and ethanol abolish the interphase diminution of pain in the formalin test: evidence for pain modulation by GABAA receptors. *Pharmacology Biochemistry and Behavior 46*: 661-666

119. Franklin, K.B.J., Biskin, R.S, and Bossert, J.M. (2002) Systemic administration of the neurosteroid, allopregnanolone, induces a conditioned place preference in rats. *Society for Neuroscience Abstracts*

120. Franzoni M.F., Morino P. (1989) The distribution of GABA-like-immunoreactive neurons in the brain of the newt, Triturus cristatus carnifex, and the green frog, Rana esculenta. *Cell and Tissue Research 255*: 155-166

121. Fudala P.J., Iwamoto E.T. (1986) Further studies on nicotine-induced conditioned place preference in the rat. *Pharmacology Biochemistry and Behavior 25*: 1041-1049

122. Gardner E.L. (2000) What we have learned about addiction from animal models of drug self-administration. *American Journal on Addictions 9*: 285-313

123. Gatto G.J., McBride W.J., Murphy J.M., Lumeng L., Li T.K. (1994) Ethanol self-infusion into the ventral tegmental area by alcohol- preferring rats. *Alcohol 11*: 557-564

124. Gerhardt S., Prowse J., Liebman J.M. (1982) Anxiolytic drugs selectively increase preferred duration of rewarding brain stimulation in a shuttlebox test. *Pharmacology Biochemistry and Behavior 16*: 795-799

125. Gerrits M.A., Patkina N., Zvartau E.E., van Ree J.M. (1995) Opioid blockade attenuates acquisition and expression of cocaine-induced place preference conditioning in rats. *Psychopharmacology 119*: 92-98

126. Gerrits M.A., Ramsey N.F., Wolterink G., van Ree J.M. (1994) Lack of evidence for an involvement of nucleus accumbens dopamine D1 receptors in the initiation of heroin self-administration in the rat. *Psychopharmacology 114*: 486-494

127. Gerrits M.A., van Ree J.M. (1996) Effect of nucleus accumbens dopamine depletion on motivational aspects involved in initiation of cocaine and heroin self-

administration in rats. *Brain Research 713*: 114-124

128. Gewiss M.V., Marley R.J., Thorndike E.B., Goldberg S.R., Schindler C.W. (1994) GABA receptor-linked chloride channels and the behavioral effects of naltrexone in rats. *Pharmacology Biochemistry and Behavior 49*: 589-597

129. Gilbert P.E., Martin W.R. (1977) Antagonism of the effects of pentobarbital in the chronic spinal dog by naltrexone. *Life Sciences 20*: 1401-1406

130. Gillette M.A., Dacheux R.F. (1995) GABA- and glycine-activated currents in the rod bipolar cell of the rabbit retina. *Journal of Neurophysiology 74*: 856-875

131. Glass M.J., Billington C.J., Levine A.S. (1999) Opioids and food intake: distributed functional neural pathways? *Neuropeptides 33*: 360-368

132. Goeders N.E., Lane J.D., Smith J.E. (1984) Self-administration of methionine enkephalin into the nucleus accumbens. *Pharmacology Biochemistry and Behavior 20*: 451-455

133. Goeders N.E., Smith J.E. (1983) Cortical dopaminergic involvement in cocaine reinforcement. *Science 221*: 773-775

134. Gong W., Neill D., Justice J.B. Jr. (1996) Conditioned place preference and locomotor activation produced by injection of psychostimulants into ventral pallidum. *Brain Research 707*: 64-74

135. Gong W., Neill D., Justice J.B. Jr. (1997) 6-Hydroxydopamine lesion of ventral pallidum blocks acquisition of place preference conditioning to cocaine. *Brain Research 754*: 103-112

136. Graeff F.G., Brandao M.L., Audi E.A., Milani H. (1986a) Role of GABA in the anti-aversive action of anxiolytics. *Advances in Biochemical Psychopharmacology 42*: 79-86

137. Graeff F.G., Brandao M.L., Audi E.A., Schutz M.T. (1986b) Modulation of the brain aversive system by GABAergic and serotonergic mechanisms. *Behavioural Brain Research 21*: 65-72

138. Grahame N.J., Cunningham C.L. (1997) Intravenous ethanol self-administration in C57BL/6J and DBA/2J mice. *Alcoholism: Clinical and Experimental Research 21*: 56-62

139. Griffiths R.R., Bigelow G., Liebson I. (1979) Human drug self-administration: double-blind comparison of pentobarbital, diazepam, chlorpromazine and placebo. *Journal of Pharmacology and Experimental Therapeutics 210*: 301-310

140. Griffiths R.R., Bigelow G.E., Liebson I. (1983) Differential effects of diazepam and pentobarbital on mood and behavior. *Archives of General Psychiatry 40*: 865-873

141. Griffiths R.R., Bigelow G.E., Liebson I., Kaliszak J.E. (1980) Drug preference in humans: double-blind choice comparison of pentobarbital, diazepam and placebo.

Journal of Pharmacology and Experimental Therapeutics 215: 649-661

142. Griffiths R.R., Lukas S.E., Bradford L.D., Brady J.V., Snell J.D. (1981) Self-injection of barbiturates and benzodiazepines in baboons. *Psychopharmacology 75:* 101-109

143. Grobin A.C., Matthews D.B., Devaud L.L., Morrow A.L. (1998) The role of GABA(A) receptors in the acute and chronic effects of ethanol. *Psychopharmacology 139*: 2-19

144. Haefely W. (1987) Structure and function of the benzodiazepine receptor. *Chimia 41*: 389-396

145. Hakan R.L. (2001) Tonic opioid inhibition of the subiculo-accumbens pathway. *Synapse 41*: 71-85

146. Harrigan S.E., Downs D.A. (1978) Self-administration of heroin, acetylmethadol, morphine, and methadone in rhesus monkeys. *Life Sciences 22*: 619-623

147. Harrison N.L., Simmonds M.A. (1983) Two distinct interactions of barbiturates and chlormethiazole with the GABAA receptor complex in rat cuneate nucleus in vitro. *British Journal of Pharmacology 80*: 387-394

148. Hays W.L.(1963) *Statistics for psychologists*. Holt, Rinehart and Winston, New York

149. Heale V., Harley C. (1990) MK-801 and AP5 impair acquisition, but not retention, of the Morris milk maze. *Pharmacology Biochemistry and Behavior 36*: 145-149

150. Heimer L., Zahm D.S., Churchill L., Kalivas P.W., Wohltmann C. (1991) Specificity in the projection patterns of accumbal core and shell in the rat. *Neuroscience 41*: 89-125

151. Hemby S.E., Jones G.H., Justice J.B. Jr., Neill D.B. (1992) Conditioned locomotor activity but not conditioned place preference following intra-accumbens infusions of cocaine. *Psychopharmacology 106*: 330-336

152. Hemby S.E., Smith J.E., Dworkin S.I. (1996) The effects of eticlopride and naltrexone on responding maintained by food, cocaine, heroin and cocaine/heroin combinations in rats *Journal of Pharmacology and Experimental Therapeutics 277*: 1247-1258

153. Herberg L.J., Williams S.F. (1983) Anti-conflict and depressant effects by GABA agonists and antagonists, benzodiazepines and non-gabergic anticonvulsants on self-stimulation and locomotor activity. *Pharmacology Biochemistry and Behavior 19*: 625-633

154. Herling S., Valentino R.J., Winger G.D. (1980) Discriminative stimulus effects of pentobarbital in pigeons. *Psychopharmacology 71*: 21-28

155. Herz A. (1997) Endogenous opioid systems and alcohol addiction. *Psychopharmacology 129*: 99-111

156. Hevers W., Luddens H. (1998) The diversity of GABAA receptors. Pharmacological and electrophysiological properties of GABAA channel subtypes. *Molecular Neurobiology 18*: 35-86

157. Heyser C.J., Roberts A.J., Schulteis G., Koob G.F. (1999) Central administration of an opiate antagonist decreases oral ethanol self-administration in rats. *Alcoholism: Clinical and Experimental Research 23*: 1468-1476

158. Hill H.E., Jones B.E., Bell E.C. (1971) State dependent control of discrimination by morphine and pentobarbital. *Psychopharmacology 22*: 305-313

159. Hill J.L., Zacny J.P. (2000) Comparing the subjective, psychomotor, and physiological effects of intravenous hydromorphone and morphine in healthy volunteers. *Psychopharmacology 152*: 31-39

160. Hiroi N., White N.M. (1990) The reserpine-sensitive dopamine pool mediates (+)-amphetamine-conditioned reward in the place preference paradigm. *Brain Research 510*: 33-42

161. Hiroi N., White N.M. (1991a) The amphetamine conditioned place preference: differential involvement of dopamine receptor subtypes and two dopaminergic terminal areas. *Brain Research 552*: 141-152

162. Hiroi N., White N.M. (1991b) The lateral nucleus of the amygdala mediates expression of the amphetamine-produced conditioned place preference. *Journal of Neuroscience 11*: 2107-2116

163. Ho I.K., Yamamoto I., Loh H.H. (1975) A model for the rapid development of dispositional and functional tolerance to barbiturates. *European Journal of Pharmacology 30*: 164-171

164. Hodge C.W., Chappelle A.M., Samson H.H. (1995) GABAergic transmission in the nucleus accumbens is involved in the termination of ethanol self-administration in rats. *Alcoholism: Clinical and Experimental Research 19*: 1486-1493

165. Hodge C.W., Cox A.A. (1998) The discriminative stimulus effects of ethanol are mediated by NMDA and GABA(A) receptors in specific limbic brain regions. *Psychopharmacology 139*: 95-107

166. Hodge C.W., Cox A.A., Bratt A.M., Camarini R., Iller K., Kelley S.P., Mehmert K.K., Nannini M.A., Olive M.F. (2001a) The discriminative stimulus properties of self-administered ethanol are mediated by GABA(A) and NMDA receptors in rats. *Psychopharmacology 154*: 13-22

167. Hodge C.W., Nannini M.A., Olive M.F., Kelley S.P., Mehmert K.K. (2001b) Allopregnanolone and pentobarbital infused into the nucleus accumbens substitute for the discriminative stimulus effects of ethanol. *Alcoholism: Clinical and Experimental Research 25*: 1441-1447

168. Hoebel B.G., Monaco A.P., Hernandez L., Aulisi E.F., Stanley B.G., Lenard L. (1983) Self-injection of amphetamine directly into the brain. *Psychopharmacology 81*: 158-163

169. Hoffman D.C. (1994) The noncompetitive NMDA antagonist MK-801 fails to block amphetamine-induced place conditioning in rats. *Pharmacology Biochemistry and Behavior 47*: 907-912

170. Hoffman D.C., Beninger R.J. (1989) The effects of selective dopamine D1 or D2 receptor antagonists on the establishment of agonist-induced place conditioning in rats. *Pharmacology Biochemistry and Behavior 33*: 273-279

171. Hyytia P., Kiianmaa K. (2001) Suppression of ethanol responding by centrally administered CTOP and naltrindole in AA and Wistar rats. *Alcoholism: Clinical and Experimental Research 25*: 25-33

172. Hyytia P., Koob G.F. (1995) GABAA receptor antagonism in the extended amygdala decreases ethanol self-administration in rats. *European Journal of Pharmacology 283*: 151-159

173. Ikemoto S., Kohl R.R., McBride W.J. (1997a) GABA(A) receptor blockade in the anterior ventral tegmental area increases extracellular levels of dopamine in the nucleus accumbens of rats. *Journal of Neurochemistry 69*: 137-143

174. Ikemoto S., Murphy J.M., McBride W.J. (1997b) Self-infusion of GABA(A) antagonists directly into the ventral tegmental area and adjacent regions. *Behavioral Neuroscience 111*: 369-380

175. Ikemoto S., Murphy J.M., McBride W.J. (1998) Regional differences within the rat ventral tegmental area for muscimol self-infusions. *Pharmacology Biochemistry and Behavior 61*: 87-92

176. Invernizzi R., Pozzi L., Samanin R. (1991) Release of dopamine is reduced by diazepam more in the nucleus accumbens than in the caudate nucleus of conscious rats. *Neuropharmacology 30*: 575-578

177. Ito T., Suzuki T., Wellman S.E., Ho I.K. (1996) Pharmacology of barbiturate tolerance/dependence: GABAA receptors and molecular aspects. *Life Sciences 59*: 169-195

178. Iwamoto E.T. (1985a) Place-aversion conditioned by phencyclidine in rats: development of tolerance and pharmacologic antagonism. *Alcohol and Drug Research 6*: 265-276

179. Iwamoto E.T. (1985b) Place-conditioning properties of mu, kappa, and sigma opioid agonists. *Alcohol and Drug Research 6*: 327-339

180. Iwamoto E.T. (1990) Nicotine conditions place preferences after intracerebral administration in rats. *Psychopharmacology 100*: 251-257

181. Jacobs M.R., Fehr K.O. (1987) *Drugs and drugs of abuse: A reference text*, 2 edn. Canadian Catalogue Publishers, Toronto

182. Jackson A., Crossman A.R. (1983) Nucleus tegmenti pedunculopontinus: efferent connections with special reference to the basal ganglia, studied in the rat by anterograde and retrograde transport of horseradish peroxidase. *Neuroscience 10*: 725-765

183. Johnson S.W., North R.A. (1992a) Opioids excite dopamine neurons by hyperpolarization of local interneurons. Journal of Neuroscience. 12: 483-488

184. Johnson S.W., North R.A. (1992b) Two types of neurone in the rat ventral tegmental area and their synaptic inputs. *Journal of Physiology 450*: 455-468

185. Jorenby D.E., Steinpreis R.E., Sherman J.E., Baker T.B. (1990) Aversion instead of preference learning indicated by nicotine place conditioning in rats. *Psychopharmacology 101*: 533-538

186. Joy R.M., Albertson T.E. (1991) Interactions of GABAA antagonists with deltamethrin, diazepam, pentobarbital, and SKF100330A in the rat dentate gyrus. *Toxicology and Applied Pharmacology 109*: 251-262

187. Kamiya Y., Andoh T., Furuya R., Hattori S., Watanabe I., Sasaki T., Ito H., Okumura F. (1999) Comparison of the effects of convulsant and depressant barbiturate stereoisomers on AMPA-type glutamate receptors. *Anesthesiology 90*:

1704-1713

188. Kamiya Y., Andoh T., Watanabe I., Higashi T., Itoh H. (2001) Inhibitory effects of barbiturates on nicotinic acetylcholine receptors in rat central nervous system neurons. *Anesthesiology 94*: 694-704

189. Kamp C.W., Morgan W.W. (1980) Some barbiturates enhance the effect of muscimol on dopamine turnover in the rat retina. *Journal of Pharmacology and Experimental Therapeutics 213*: 332-336

190. Kelsey J.E., Carlezon W.A. Jr., Falls W.A. (1989) Lesions of the nucleus accumbens in rats reduce opiate reward but do not alter context-specific opiate tolerance. *Behavioral Neuroscience 103*: 1327-1334

191. Khanna J.M., Kalant H., Le A.D., Mayer J., LeBlanc A.E. (1980) Effect of p-chlorophenylalanine on the acquisition of tolerance to the hypnotic effects of pentobarbital, barbital, and ethanol. *Canadian Journal of Physiology and Pharmacology 58*: 1031-1041

192. Kim H.S., Park W.K., Jang C.G., Oh K.W., Kong J.Y., Oh S., Rheu H.M., Cho D.H., Kang S.Y. (1997) Blockade by naloxone of cocaine-induced hyperactivity, reverse tolerance and conditioned place preference in mice. *Behavioural Brain Research 85*: 37-46

193. Klitenick M.A., DeWitte P., Kalivas P.W. (1992) Regulation of somatodendritic dopamine release in the ventral tegmental area by opioids and GABA: an in vivo microdialysis study. *Journal of Neuroscience 12*: 2623-2632

194. Kohl R.R., Katner J.S., Chernet E., McBride W.J. (1998) Ethanol and negative feedback regulation of mesolimbic dopamine release in rats. *Psychopharmacology 139*: 79-85

195. Koistinen M., Tuomainen P., Hyytia P., Kiianmaa K. (2001) Naltrexone suppresses ethanol intake in 6-hydroxydopamine-treated rats. *Alcoholism: Clinical and Experimental Research 25*: 1605-1612

196. Koltchine V.V., Ye Q., Finn S.E., Harrison N.L. (1996) Chimeric GABAA/glycine receptors: expression and barbiturate pharmacology. *Neuropharmacology 35*: 1445-1456

197. Koob G.F. (1992) Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends in Pharmacological Sciences 13*: 177-184

198. Koob G.F. (1999) The role of the striatopallidal and extended amygdala systems in drug addiction. *Annals of the New York Academy of Sciences 877*: 445-460

199. Koob G.F., Goeders N.E. (1989) Neuroanatomical substrates of drug self-administration. In: Liebman JM, Cooper SJ (eds) *The neuropharmacological basis of reward.* Oxford University Press, New York, pp 214-263

200. Koob G.F., Pettit H.O., Ettenberg A., Bloom F.E. (1984) Effects of opiate antagonists and their quaternary derivatives on heroin self-administration in the rat. *Journal of Pharmacology and Experimental Therapeutics 229*: 481-486

201. Koob G.F., Roberts A.J., Schulteis G., Parsons L.H., Heyser C.J., Hyytia P., Merlo-Pich E., Weiss F. (1998) Neurocircuitry targets in ethanol reward and dependence. *Alcoholism: Clinical and Experimental Research 22*: 3-9

202. Koob G.F., Weiss F. (1990) Pharmacology of drug self-administration. *Alcohol 7*: 193-197

203. Kornetsky C., Esposito R.U., McLean S., Jacobson J.O. (1979) Intracranial self-stimulation thresholds: a model for the hedonic effects of drugs of abuse. *Archives of General Psychiatry 36*: 289-292

204. Kubota T., Anzawa N., Hirota K., Yoshida H., Kushikata T., Matsuki A. (1999) Effects of ketamine and pentobarbital on noradrenaline release from the medial prefrontal cortex in rats. *Canadian Journal of Anaesthesia 46*: 388-392

205. Lamb R.J., Preston K.L., Schindler C.W., Meisch R.A., Davis F., Katz J.L., Henningfield J.E., Goldberg S.R. (1991) The reinforcing and subjective effects of morphine in post-addicts: a dose-response study. *Journal of Pharmacology and Experimental Therapeutics 259*: 1165-1173

206. Laviolette S.R., Nader K., van der kooy, D. (2002) Motivational state determines

the functional role of the mesolimbic dopamine system in the mediation of opiate reward processes. *Behavioural Brain Research 129*: 17-29

207. Laviolette S.R., van der Kooy D. (2001) GABA(A) receptors in the ventral tegmental area control bidirectional reward signalling between dopaminergic and non-dopaminergic neural motivational systems. *European Journal of Neuroscience 13*: 1009-1015

208. Leeb-Lundberg F., Snowman A., Olsen R.W. (1980) Barbiturate receptor sites are coupled to benzodiazepine receptors. *Proceedings of the National Academy of Sciences of the United States of America 77*: 7468-7472

209. Lemaire G.A., Meisch R.A. (1984) Pentobarbital self-administration in rhesus monkeys: drug concentration and fixed-ratio size interactions. *Journal of the Experimental Analysis of Behavior 42*: 37-49

210. Leri F., Franklin K.B.J. (2000a) Diazepam in the ventral striatum dissociates dopamine-dependent and dopamine-independent place conditioning. *Neuroreport 11*: 2553-2557

211. Leri F., Franklin K.B.J. (2000b) Effects of diazepam on conditioned place preference induced by morphine or amphetamine in the rat. *Psychopharmacology 150*: 351-360

212. Leri F., Franklin K.B.J. (2000c) Diazepam modifies the effect of pedunculopontine lesions on morphine but not on amphetamine conditioned place preference.

Behavioural Brain Research 117: 21-27

213. Letterman L., Markowitz J.S.. (1999) Gabapentin: a review of published experience in the treatment of bipolar disorder and other psychiatric conditions. *Pharmacotherapy 19*: 565-572

214. Lew G., Parker L.A. (1998) Pentobarbital-induced place aversion learning. *Animal Learning and Behavior 26*: 219-224

215. Liao R.M., Chang Y.H., Wang S.H., Lan C.H. (2000) Distinct accumbal subareas are involved in place conditioning of amphetamine and cocaine. *Life Sciences 67*: 2033-2043

216. Lidbrink P., Corrodi H., Fuxe K., Olson L. (1972) Barbiturates and meprobamate: decreases in cathecholamine turnover of central dopamine and noradrenaline neuronal systems and the influence of immobilization stress. *Brain Research 45*: 507-524

217. Lillie R.D. (1965) Buffers and buffer tables, normal acids and alkalies. In: *Histopathologic technic and practical histochemistry*. McGraw-Hill, New York,

218. Lindvall O., Bjorklund A. (1974) The organization of the ascending catecholamine neuron systems in the rat brain as revealed by the glyoxylic acid fluorescence method. *Acta Physiologica Scandinavica Suppl 412*: 1-48

219. Liu X., Weiss F. (2002) Reversal of ethanol-seeking behavior by D1 and D2

antagonists in an animal model of relapse: differences in antagonist potency in previously ethanol-dependent versus nondependent rats. *Journal of Pharmacology and Experimental Therapeutics 300*: 882-889

220. Lohse M.J., Klotz K.N., Jakobs K.H., Schwabe U. (1985) Barbiturates are selective antagonists at A1 adenosine receptors. *Journal of Neurochemistry 45*: 1761-1770

221. Lorens S.A., Sainati S.M. (1978) Naloxone blocks the excitatory effect of ethanol and chlordiazepoxide on lateral hypothalamic self-stimulation behavior. *Life Sciences 23*: 1359-1364

222. Lovick T.A., Paul N.L. (1999) Co-localization of GABA with nicotinamide adenine dinucleotide phosphate-dependent diaphorase in neurones in the dorsolateral periaqueductal grey matter of the rat. *Neuroscience Letters 272*: 167-170

223. Lyness W.H., Brezenoff H.E., Mycek M.J. (1979a) Cross-tolerance to centrally injected barbiturates. *European Journal of Pharmacology 54*: 319-330

224. Lyness W.H., Friedle N.M., Moore K.E. (1979b) Destruction of dopaminergic nerve terminals in nucleus accumbens: effect on d-amphetamine self-administration. *Pharmacology Biochemistry and Behavior 11*: 553-556

225. Macdonald R.L., Olsen R.W. (1994) GABAA receptor channels. *Annual Review of Neuroscience 17*: 569-602

226. Mackey W.B., van der Kooy D. (1985) Neuroleptics block the positive reinforcing effects of amphetamine but not of morphine as measured by place conditioning. *Pharmacology Biochemistry and Behavior 22*: 101-105

227. Malcangio M., Malmberg-Aiello P., Giotti A., Ghelardini C., Bartolini A. (1992) Desensitization of GABAB receptors and antagonism by CGP 35348, prevent bicuculline- and picrotoxin-induced antinociception. *Neuropharmacology 31*: 783-791

228. Manning B.H., Franklin K.B.J. (1998) Morphine analgesia in the formalin test: reversal by microinjection of quaternary naloxone into the posterior hypothalamic area or periaqueductal gray. *Behavioural Brain Research 92*: 97-102

229. Mansour A., Fox C.A., Burke S., Akil H., Watson S.J. (1995) Immunohistochemical localization of the cloned mu opioid receptor in the rat CNS. *Journal of Chemical Neuroanatomy 8*: 283-305

230. Marglin S.H., Milano W.C., Mattie M.E., Reid L.D. (1989) PCP and conditioned place preferences. *Pharmacology Biochemistry and Behavior 33*: 281-283

231. Mariathasan E.A., Stolerman I.P. (1994) Functional relationships, previous history and the discrimination of a drug mixture in rats. *Drug and Alcohol Dependence 35*: 117-125

232. Marsch L.A., Bickel W.K., Badger G.J., Rathmell J.P., Swedberg M.D., Jonzon B., Norsten-Hoog C. (2001) Effects of infusion rate of intravenously administered morphine on physiological, psychomotor, and self-reported measures in humans. *Journal of Pharmacology and Experimental Therapeutics 299*: 1056-1065

233. Matsuzawa S., Suzuki T., Misawa M. (2000) Ethanol, but not the anxiolytic drugs buspirone and diazepam, produces a conditioned place preference in rats exposed to conditioned fear stress. *Pharmacology Biochemistry and Behavior 65*: 81-88

234. Mayer L.A., Parker L.A. (1993) Rewarding and aversive properties of IP and SC cocaine: assessment by place and taste conditioning. *Psychopharmacology 112*: 189-194

235. McBride W.J., Li T.K. (1998) Animal models of alcoholism: neurobiology of high alcohol-drinking behavior in rodents. *Critical Reviews in Neurobiology 12*: 339-369

236. McBride W.J., Murphy J.M., Gatto G.J., Levy A.D., Yoshimoto K., Lumeng L., Li T.K. (1993) CNS mechanisms of alcohol self-administration. *Alcohol and Alcoholism Suppl 2*: 463-467

237. McBride W.J., Murphy J.M., Ikemoto S. (1999) Localization of brain reinforcement mechanisms: intracranial self-administration and intracranial place-conditioning studies. *Behavioural Brain Research 101*: 129-152

238. McGregor A., Baker G., Roberts D.C. (1996) Effect of 6-hydroxydopamine lesions of the medial prefrontal cortex on intravenous cocaine self-administration under a progressive ratio schedule of reinforcement. *Pharmacology Biochemistry and Behavior 53*: 5-9

239. McLeod D.R., Griffiths R.R. (1983) Human progressive-ratio performance: maintenance by pentobarbital. *Psychopharmacology 79*: 4-9

240. McMillan D.E., Hardwick W.C., Li M. (2001) Discrimination of pentobarbital doses and drug mixtures under fixed- ratio and fixed-interval reinforcement schedules. *Behavioural Pharmacology 12*: 195-208

241. Mehta A.K., Ticku M.K. (1986) Comparison of anticonvulsant effect of pentobarbital and phenobarbital against seizures induced by maximal electroshock and picrotoxin in rats. *Pharmacology Biochemistry and Behavior 25*: 1059-1065

242. Melendez R.I., Rodd-Henricks Z.A., Engleman E.A., Li T.K., McBride W.J., Murphy J.M. (2002) Microdialysis of dopamine in the nucleus accumbens of alcohol-preferring (P) rats during anticipation and operant self-administration of ethanol. *Alcoholism: Clinical and Experimental Research 26*: 318-325

243. Mereu G., Gessa G.L. (1985) Low doses of ethanol inhibit the firing of neurons in the substantia nigra, pars reticulata: a GABAergic effect? *Brain Research 360*: 325-330

244. Meririnne E., Kankaanpaa A., Lillsunde P., Seppala T. (1999) The effects of diazepam and zolpidem on cocaine- and amphetamine-induced place preference. *Pharmacology Biochemistry and Behavior 62*: 159-164

245. Mestdagh N., Wulfert E. (1999) Bicuculline increases Ca2+ transients in rat cerebellar granule cells through non-GABA(A) receptor associated mechanisms. *Neuroscience Letters 265*: 95-98

246. Millan M.J. (2002) Descending control of pain. *Progress in Neurobiology 66*: 355-474

247. Mintzer M.Z., Guarino J., Kirk T., Roache J.D., Griffiths R.R. (1997) Ethanol and pentobarbital: comparison of behavioral and subjective effects in sedative drug abusers. *Experimental and Clinical Psychopharmacology 5*: 203-215

248. Miyamoto Y., Noda Y., Komori Y., Sugihara H., Furukawa H., Nabeshima T. (2000) Involvement of nitric oxide in phencyclidine-induced place aversion and preference in mice. *Behavioural Brain Research 116*: 187-196

249. Mondadori C., Weiskrantz L., Buerki H., Petschke F., Fagg G.E. (1989) NMDA receptor antagonists can enhance or impair learning performance in animals. *Experimental Brain Research 75*: 449-456

250. Morency M.A., Beninger R.J. (1986) Dopaminergic substrates of cocaine-induced place conditioning. *Brain Research 399*: 33-41

251. Morgan M.M., Whitney P.K., Gold M.S. (1998) Immobility and flight associated with antinociception produced by activation of the ventral and lateral/dorsal regions of the rat periaqueductal gray. *Brain Research 804*: 159-166

252. Motta V., Brandao M.L. (1993) Aversive and antiaversive effects of morphine in the dorsal periaqueductal gray of rats submitted to the elevated plus-maze test. *Pharmacology Biochemistry and Behavior 44*: 119-125

253. Mucha R.F., Iversen S.D. (1984) Reinforcing properties of morphine and naloxone revealed by conditioned place preferences: a procedural examination. *Psychopharmacology 82*: 241-247

254. Mucha R.F., van der Kooy D., O'Shaughnessy M., Bucenieks P. (1982) Drug reinforcement studied by the use of place conditioning in rat. *Brain Research 243*: 91-105

255. Nabeshima T., Fujimori K., Ho I.K. (1981) Effect of acute or chronic pentobarbital administration on the steady state levels and the turnover rates of catecholamines in discrete brain areas of mice. *Progress in Neuropsychopharmacology 5*: 121-128

256. Nader K., van der Kooy D. (1997) Deprivation state switches the neurobiological substrates mediating opiate reward in the ventral tegmental area. *Journal of Neuroscience 17*: 383-390

257. Negus S.S., Henriksen S.J., Mattox A., Pasternak G.W., Portoghese P.S., Takemori A.E., Weinger M.B., Koob G.F. (1993) Effect of antagonists selective for mu, delta and kappa opioid receptors on the reinforcing effects of heroin in rats. *Journal of Pharmacology and Experimental Therapeutics 265*: 1245-1252

258. Neisewander J.L., McDougall S.A., Bowling S.L., Bardo M.T. (1990) Conditioned taste aversion and place preference with buspirone and gepirone. *Psychopharmacology 100*: 485-490

259. Nestoros J.N. (1984) Gabaergic mechanisms and anxiety: an overview and a new neurophysiological model. *Canadian Journal of Psychiatry 29*: 520-529

260. Nowak K.L., McBride W.J., Lumeng L., Li T.K., Murphy J.M. (1998) Blocking GABA(A) receptors in the anterior ventral tegmental area attenuates ethanol intake of the alcohol-preferring P rat. *Psychopharmacology 139*: 108-116

261. Okamoto M., Hinman D.J. (1984) Physical dependence produced by long duration, low dose chronic barbital treatment. *Substance and Alcohol Actions/Misuse 5*: 97-103

262. Olds J. (1956) A preliminary mapping of electrical reinforcing effects in the rat brain. *Journal of Comparative and Physiological Psychology 49:* 281-285

263. Olds J., Milner P.M. (1954) Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *Journal of Comparative and*

Physiological Psychology 47: 419-427

264. Olds M.E. (1982) Reinforcing effects of morphine in the nucleus accumbens. *Brain Research 237*: 429-440

265. Olds M.E., Ito M. (1973) Effects of chlorpromazine, chlordiazepoxide and pentobarbital on neuronal excitability in the medial forebrain bundle during self-stimulation behavior. *Neuropharmacology 12*: 1117-1133

266. Oliveira M.A., Prado W.A. (2001) Role of PAG in the antinociception evoked from the medial or central amygdala in rats. *Brain Research Bulletin 54*: 55-63

267. Olmstead M.C., Franklin K.B.J. (1996) Differential effects of ventral striatal lesions on the conditioned place preference induced by morphine or amphetamine. *Neuroscience 71*: 701-708

268. Olmstead M.C., Franklin K.B.J. (1997a) The development of a conditioned place preference to morphine: effects of lesions of various CNS sites. *Behavioral Neuroscience 111*: 1313-1323

269. Olmstead M.C., Franklin K.B.J. (1997b) The development of a conditioned place preference to morphine: effects of microinjections into various CNS sites. *Behavioral Neuroscience 111*: 1324-1334

270. Olsen R.W. (1988) Barbiturates. *International Anesthesiology Clinics 26*: 254-261

271. Olsen R.W., Snowman A.M. (1982) Chloride-dependent enhancement by barbiturates of gamma-aminobutyric acid receptor binding. *Journal of Neuroscience 2*: 1812-1823

272. Overton D.A. (1966) State-dependent learning produced by depressant and atropine-like drugs. *Psychopharmacology 10*: 6-31

273. Pain L., Oberling P., Sandner G., Di Scala G. (1997) Effect of midazolam on propofol-induced positive affective state assessed by place conditioning in rats. *Anesthesiology 87*: 935-943

274. Panos J.J., Rademacher D.J., Renner S.L., Steinpreis R.E. (1999) The rewarding properties of NMDA and MK-801 (dizocilpine) as indexed by the conditioned place preference paradigm. *Pharmacology Biochemistry and Behavior 64*: 591-595

275. Parker L.A. (1992) Place conditioning in a three- or four-choice apparatus: role of stimulus novelty in drug-induced place conditioning. *Behavioral Neuroscience 106*: 294-306

276. Paul M.S. (1995) GABA and glycine. In: Bloom FE, Kupfer DJ (eds) *Psychopharmacology: the fourth generation of progress*. Raven Press, New York, pp 87-94

277. Paxinos G. (1995) *The rat nervous system*. 2 edn. Academic Press, San Diego, CA

278. Paxinos G., Watson C. (1998) *The rat brain in stereotaxic coordinates*. 4 edn. Academic Press, San Diego, CA

279. Petry N.M. (1997) Benzodiazepine-GABA modulation of concurrent ethanol and sucrose reinforcement in the rat. *Experimental and Clinical Psychopharmacology 5*: 183-194

280. Pettit H.O., Batsell W.R., Mueller K. (1989) Triazolam attenuates amphetamine but not morphine conditioned place preferences. *Psychopharmacology 98*: 483-486

281. Pettit H.O., Ettenberg A., Bloom F.E., Koob G.F. (1984) Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. *Psychopharmacology 84*: 167-173

282. Phillips A.G., Blaha C.D., Fibiger H.C. (1989) Neurochemical correlates of brain-stimulation reward measured by ex vivo and in vivo analyses. *Neuroscience and Biobehavioral Reviews 13*: 99-104

283. Phillips A.G., Fibiger H.C. (1987) Anatomical and neurochemical substrates of drug reward determined by the conditioned place preference technique. In: Bozarth MA (ed) *Methods of assessing the reinforcing properties of drugs*. Springer-Verlag, New York, pp 275-290

284. Phillips A.G., Fibiger H.C. (1989) Neuroanatomical bases of intracranial self-stimulation: untangling the Gordian knot. In: Liebman JM, Cooper SJ (eds) *The*

neuropharmacological basis of reward. Oxford University Press, New York, pp 66-105

285. Phillips A.G., LePiane F.G. (1980) Reinforcing effects of morphine microinjection into the ventral tegmental area. *Pharmacology Biochemistry and Behavior 12*: 965-968

286. Phillips G.D., Robbins T.W., Everitt B.J. (1994) Bilateral intra-accumbens self-administration of d-amphetamine: antagonism with intra-accumbens SCH-23390 and sulpiride. *Psychopharmacology 114*: 477-485

287. Pickens R., Cunningham M.R., Heston L.L., Eckert E., Gustafson L.K. (1977) Dose preference during pentobarbital self-administration by humans. *Journal of Pharmacology and Experimental Therapeutics 203*: 310-318

288. Pickens R., Muchow D., DeNoble V. (1981) Methohexital-reinforced responding in rats: effects of fixed ratio size and injection dose. *Journal of Pharmacology and Experimental Therapeutics 216*: 205-209

289. Piepponen T.P., Kivastik T., Katajamaki J., Zharkovsky A., Ahtee L. (1997) Involvement of opioid mu 1 receptors in morphine-induced conditioned place preference in rats. *Pharmacology Biochemistry and Behavior 58*: 275-279

290. Planeta C.S., Aizenstein M.L., DeLucia R. (1995) Reinforcing properties of fencamfamine: involvement of dopamine and opioid receptors. *Pharmacology*

Biochemistry and Behavior 50: 35-40

291. Pontieri F.E., Tanda G., Di Chiara G. (1995) Intravenous cocaine, morphine, and amphetamine preferentially increase extracellular dopamine in the "shell" as compared with the "core" of the rat nucleus accumbens. *Proceedings of the National Academy of Sciences of the United States of America 92*: 12304-12308

292. Predy P.A., Kokkindis L. (1984) Sensitization to the effects of repeated amphetamine administration on intracranial self-stimulation: evidence for changes in reward processes. *Behavioural Brain Research 13*: 251-259

293. Pritchett D.B., Sontheimer H., Shivers B.D., Ymer S., Kettenmann H., Schofield P.R., Seeburg P.H. (1989) Importance of a novel GABAA receptor subunit for benzodiazepine pharmacology. *Nature 338:* 582-585

294. Rademacher D.J., Anders K.A., Thompson K.J., Steinpreis R.E. (2000) The failure of some rats to acquire intravenous cocaine self-administration is attributable to conditioned place aversion. *Behavioural Brain Research 117*: 13-19

295. Rassnick S., Pulvirenti L., Koob G.F. (1992) Oral ethanol self-administration in rats is reduced by the administration of dopamine and glutamate receptor antagonists into the nucleus accumbens. *Psychopharmacology 109*: 92-98

296. Rassnick S., Stinus L., Koob G.F. (1993) The effects of 6-hydroxydopamine lesions of the nucleus accumbens and the mesolimbic dopamine system on oral self-

administration of ethanol in the rat. *Brain Research 623*: 16-24

297. Rastogi S.K., Ticku M.K. (1985) Involvement of a GABAergic mechanism in the anticonvulsant effect of pentobarbital against maximal electroshock-induced seizures in rats. *Pharmacology Biochemistry and Behavior 22*: 141-146

298. Reynolds S.M., Berridge K.C. (2002) Positive and negative motivation in nucleus accumbens shell: bivalent rostrocaudal gradients for GABA-elicited eating, taste "liking"/"disliking" reactions, place preference/avoidance, and fear. *Journal of Neuroscience 22*: 7308-7320

299. Rho J.M., Donevan S.D., Rogawski M.A. (1996) Direct activation of GABAA receptors by barbiturates in cultured rat hippocampal neurons. *Journal of Physiology 497*: 509-522

300. Risinger F.O., Dickinson S.D., Cunningham C.L. (1992) Haloperidol reduces ethanol-induced motor activity stimulation but not conditioned place preference. *Psychopharmacology 107*: 453-456

301. Risinger F.O., Oakes R.A. (1995) Nicotine-induced conditioned place preference and conditioned place aversion in mice. *Pharmacology Biochemistry and Behavior 51*: 457-461

302. Ritz M.C., Kuhar M.J. (1993) Psychostimulant drugs and a dopamine hypothesis regarding addiction: update on recent research. *Biochemical Society Symposia 59*:

51-64

303. Roberts A.J., Cole M., Koob G.F. (1996) Intra-amygdala muscimol decreases operant ethanol self-administration in dependent rats. *Alcoholism: Clinical and Experimental Research 20*: 1289-1298

304. Roberts D.C., Corcoran M.E., Fibiger H.C. (1977) On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. *Pharmacology Biochemistry and Behavior 6*: 615-620

305. Roberts D.C., Koob G.F. (1982) Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. *Pharmacology Biochemistry and Behavior 17*: 901-904

306. Roberts M.R., Eng-Bourquin J. (1995) Status epilepticus in children. *Emergency Medicine Clinics of North America 13*: 489-507

307. Robinson T.E., Camp D.M. (1991) On the preferential release of mesolimbic dopamine by amphetamine. *Neuropsychopharmacology 5*: 243-247

308. Roca D.J., Schiller G.D., Friedman L., Rozenberg I., Gibbs T.T., Farb D.H. (1990) gamma-Aminobutyric acidA receptor regulation in culture: altered allosteric interactions following prolonged exposure to benzodiazepines, barbiturates, and methylxanthines. *Molecular Pharmacology 37*: 710-719

309. Rodd-Henricks Z.A., McKinzie D.L., Crile R.S., Murphy J.M., McBride W.J. (2000) Regional heterogeneity for the intracranial self-administration of ethanol within the ventral tegmental area of female Wistar rats. *Psychopharmacology 149:* 217-224

310. Rodefer J.S., Campbell U.C., Cosgrove K.P., Carroll M.E. (1999) Naltrexone pretreatment decreases the reinforcing effectiveness of ethanol and saccharin but not PCP or food under concurrent progressive-ratio schedules in rhesus monkeys. *Psychopharmacology 141*: 436-446

311. Rompre P.P., Wise R.A. (1989) Opioid-neuroleptic interaction in brainstem self-stimulation. *Brain Research 477*: 144-151

312. Rossetti Z.L., Hmaidan Y., Gessa G.L. (1992) Marked inhibition of mesolimbic dopamine release: a common feature of ethanol, morphine, cocaine and amphetamine abstinence in rats. *European Journal of Pharmacology 221*: 227-234

313. Roth R.H. (1984) CNS dopamine autoreceptors: distribution, pharmacology, and function. *Annals of the New York Academy of Sciences 430*: 27-53

314. Rush C.R., Ali J.A. (1999) Naltrexone does not attenuate the acute behavioral effects of ethanol or pentobarbital in humans. *Behavioural Pharmacology 10*: 401-413

315. Russo H., Bressolle F. (1998) Pharmacodynamics and pharmacokinetics of thiopental. *Clinical Pharmacokinetics 35*: 95-134

316. Salamone J.D. (1991) Behavioral pharmacology of dopamine systems: a new synthesis. In: Willner P, Scheel-Kruger J (eds) *The mesolimbic dopamine system: From motivation to action*. John Wiley & Sons, New York, pp 599-613

317. Samson H.H., Doyle T.F. (1985) Oral ethanol self-administration in the rat: effect of naloxone. *Pharmacology Biochemistry and Behavior 22*: 91-99

318. Sanna E., Garau F., Harris R.A. (1995) Novel properties of homomeric beta 1 gamma-aminobutyric acid type A receptors: actions of the anesthetics propofol and pentobarbital. *Molecular Pharmacology 47*: 213-217

319. Sawynok J., Pinsky C., LaBella F.S. (1979) On the specificity of naloxone as an opiate antagonist. *Life Sciences 25*: 1621-1632

320. Schaefer G.J., Michael R.P. (1987) Ethanol and current thresholds for brain self-stimulation in the lateral hypothalamus of the rat. *Alcohol 4*: 209-213

321. Schaefer G.J., Michael R.P. (1988) An analysis of the effects of amphetamine on brain self-stimulation behavior. *Behavioural Brain Research 29*: 93-101

322. Scheel-Kruger J., Willner P. (1991) The mesolimbic system: Principles of operation. In: Willner P, Scheel-Kruger J (eds) *The mesolimbic dopamine system: From motivation to action*. John Wiley & Sons, New York, pp 559-597

323. Schildein S., Agmo A., Huston J.P., Schwarting R.K. (1998) Intraaccumbens injections of substance P, morphine and amphetamine: effects on conditioned place preference and behavioral activity. *Brain Research 790*: 185-194

324. Schulz D.W., Macdonald R.L. (1981) Barbiturate enhancement of GABA-mediated inhibition and activation of chloride ion conductance: correlation with anticonvulsant and anesthetic actions. *Brain Research 209*: 177-188

325. Seeger T.F., Carlson K.R., Nazzaro J.M. (1981) Pentobarbital induces a naloxone-reversible decrease in mesolimbic self-stimulation threshold. *Pharmacology Biochemistry and Behavior 15*: 583-586

326. Self D.W., Stein L. (1993) Pertussis toxin attenuates intracranial morphine self-administration. *Pharmacology Biochemistry and Behavior 46*: 689-695

327. Seutin V., Scuvee-Moreau J., Dresse A. (1997) Evidence for a non-GABAergic action of quaternary salts of bicuculline on dopaminergic neurones. *Neuropharmacology 36*: 1653-1657

328. Shekhar A., DiMicco J.A. (1987) Defense reaction elicited by injection of GABA antagonists and synthesis inhibitors into the posterior hypothalamus in rats. *Neuropharmacology 26*: 407-417

329. Shekhar A., Hingtgen J.N., DiMicco J.A. (1987) Selective enhancement of shock avoidance responding elicited by GABA blockade in the posterior hypothalamus of

rats. *Brain Research 420*: 118-128

330. Shekhar A., Hingtgen J.N., DiMicco J.A. (1990) GABA receptors in the posterior hypothalamus regulate experimental anxiety in rats. *Brain Research 512*: 81-88

331. Shimosato K., Ohkuma S. (2000) Simultaneous monitoring of conditioned place preference and locomotor sensitization following repeated administration of cocaine and methamphetamine. *Pharmacology Biochemistry and Behavior 66*: 285-292

332. Shippenberg T.S., Bals-Kubik R., Herz A. (1993) Examination of the neurochemical substrates mediating the motivational effects of opioids: role of the mesolimbic dopamine system and D-1 vs. D-2 dopamine receptors. *Journal of Pharmacology and Experimental Therapeutics 265*: 53-59

333. Simson P.E., Criswell H.E., Johnson K.B., Hicks R.E., Breese G.R. (1991) Ethanol inhibits NMDA-evoked electrophysiological activity in vivo. *Journal of Pharmacology and Experimental Therapeutics 257*: 225-231

334. Smith A.J., Alder L., Silk J., Adkins C., Fletcher A.E., Scales T., Kerby J., Marshall G., Wafford K.A., McKernan R.M., Atack J.R. (2001) Effect of alpha subunit on allosteric modulation of ion channel function in stably expressed human recombinant gamma-aminobutyric acid(A) receptors determined using (36)Cl ion flux. *Molecular Pharmacology 59*: 1108-1118

335. Smith G.B., Olsen R.W. (1995) Functional domains of GABAA receptors. *Trends in Pharmacological Sciences 16*: 162-168

336. Smith S.G., Davis W.M. (1974) Intravenous alcohol self-administration in the rat. *Pharmacological Research Communications 6*: 379-402

337. Soderpalm B., Svensson A.I. (1999) Naloxone reverses disinhibitory/aggressive behavior in 5,7-DHT-lesioned rats; involvement of GABA(A) receptor blockade? *Neuropharmacology 38*: 1851-1859

338. Spyraki C., Fibiger H.C., Phillips A.G. (1982a) Cocaine-induced place preference conditioning: lack of effects of neuroleptics and 6-hydroxydopamine lesions. *Brain Research 253*: 195-203

339. Spyraki C., Fibiger H.C., Phillips A.G. (1982b) Dopaminergic substrates of amphetamine-induced place preference conditioning. *Brain Research 253*: 185-193

340. Spyraki C., Kazandjian A., Varonos D. (1985) Diazepam-induced place preference conditioning: appetitive and antiaversive properties. *Psychopharmacology 87*: 225-232

341. Stein L., Ray O.S. (1960) Brain stimulation reward 'thresholds' self-determined in rat. *Psychopharmacology 1*: 251-256

342. Stellar J.R., Corbett D. (1989) Regional neuroleptic microinjections indicate a role for nucleus accumbens in lateral hypothalamic self-stimulation reward. *Brain*

Research 477: 126-143

343. Stellar J.R., Kelley A.E., Corbett D. (1983) Effects of peripheral and central dopamine blockade on lateral hypothalamic self-stimulation: evidence for both reward and motor deficits. *Pharmacology Biochemistry and Behavior 18*: 433-442

344. Sugita S., Johnson S.W., North R.A. (1992) Synaptic inputs to GABAA and GABAB receptors originate from discrete afferent neurons. *Neuroscience Letters 134*: 207-211

345. Svensson A.I., Berntsson A., Eirefelt M., Soderpalm B. (2000) Naloxone antagonizes GABA(A)/benzodiazepine receptor function in rat corticohippocampal synaptoneurosomes. *Journal of Neural Transmission - General Section 107*: 261-270

346. Swerdlow N.R., Koob G.F. (1984) Restrained rats learn amphetamine-conditioned locomotion, but not place preference. *Psychopharmacology 84*: 163-166

347. Thompson S.A., Whiting P.J., Wafford K.A. (1996) Barbiturate interactions at the human GABAA receptor: dependence on receptor subunit combination. *British Journal of Pharmacology 117*: 521-527

348. Thorndike E.L. (1965) *Animal intelligence*. Hafner Publishing, New York

349. Tomaz C.A., Ventura D.F., Leite J.R. (1982) Effects of sodium barbitone on learning and memory-storage of an appetitive and an aversive task. *Pharmacology*

Biochemistry and Behavior 17: 909-913

350. Trifiletti R.R., Snowman A.M., Snyder S.H. (1984) Barbiturate recognition site on the GABA/benzodiazepine receptor complex is distinct from the picrotoxinin/TBPS recognition site. *European Journal of Pharmacology 106*: 441-447

351. Trifunovic R.D., Brodie M.S. (1996) The effects of clomipramine on the excitatory action of ethanol on dopaminergic neurons of the ventral tegmental area in vitro. *Journal of Pharmacology and Experimental Therapeutics 276*: 34-40

352. Trujillo K.A., Akil H. (1995) Excitatory amino acids and drugs of abuse: a role for N-methyl-D-aspartate receptors in drug tolerance, sensitization and physical dependence. *Drug and Alcohol Dependence 38*: 139-154

353. Trujillo K.A., Belluzzi J.D., Stein L. (1991) Naloxone blockade of amphetamine place preference conditioning. *Psychopharmacology 104*: 265-274

354. Tsuji M., Nakagawa Y., Ishibashi Y., Yoshii T., Takashima T., Shimada M., Suzuki T. (1996) Activation of ventral tegmental GABAB receptors inhibits morphine-induced place preference in rats. *European Journal of Pharmacology 313*: 169-173

355. Tzschentke T.M. (1998) Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. *Progress in Neurobiology 56*: 613-672

356. Ueno S., Bracamontes J., Zorumski C., Weiss D.S., Steinbach J.H. (1997) Bicuculline and gabazine are allosteric inhibitors of channel opening of the GABAA receptor. *Journal of Neuroscience 17*: 625-634

357. United States Congress and Senate (1973) Barbiturate abuse in the United States, 1973. *Hearing... Ninety-third congress, first session, on examination of proposed actions to combat barbiturate abuse in the United States, 1973*. Washington

358. Uusi-Oukari M., Korpi E.R. (1992) Functional properties of GABAA receptors in two rat lines selected for high and low alcohol sensitivity. *Alcohol 9*: 261-269

359. Vaccarino F.J., Pettit H.O., Bloom F.E., Koob G.F. (1985) Effects of intracerebroventricular administration of methyl naloxonium chloride on heroin self-administration in the rat. *Pharmacology Biochemistry and Behavior 23*: 495-498

360. van der Kooy D. (1987) Place conditioning: A simple and effective method for assessing the motivational properties of drugs. In: Bozarth MA (ed) *Methods of assessing the reinforcement properties of abused drugs*. Springer-Verlag, New York, pp 229-240

361. van der Kooy D., Mucha R.F., O'Shaughnessy M., Bucenieks P. (1982) Reinforcing effects of brain microinjections of morphine revealed by conditioned place preference. *Brain Research 243*: 107-117

362. van der Kooy D., O'Shaughnessy M., Mucha R.F., Kalant H. (1983) Motivational properties of ethanol in naive rats as studied by place conditioning. *Pharmacology Biochemistry and Behavior 19*: 441-445

363. van Ree J.M., Gerrits M.A., Vanderschuren L.J. (1999) Opioids, reward and addiction: An encounter of biology, psychology, and medicine. *Pharmacological Reviews 51*: 341-396

364. van Ree J.M., Ramsey N. (1987) The dopamine hypothesis of opiate reward challenged. *European Journal of Pharmacology 134*: 239-243

365. Vanover K.E., Wenger G.R., Woolverton W.L. (1989) Self-administration of the isomers of pentobarbital and secobarbital by rhesus monkeys. *Pharmacology Biochemistry and Behavior 34*: 669-671

366. Veenman C.L., Reiner A. (1994) The distribution of GABA-containing perikarya, fibers, and terminals in the forebrain and midbrain of pigeons, with particular reference to the basal ganglia and its projection targets. *Journal of Comparative Neurology 339*: 209-250

367. Wagner S., Castel M., Gainer H., Yarom Y. (1997) GABA in the mammalian suprachiasmatic nucleus and its role in diurnal rhythmicity. *Nature 387*: 598-603

368. Wahlstrom G. (1979) Some aspects of the changes induced by chronic barbital treatments in the male rat. *Drug and Alcohol Dependence 4*: 221-233

369. Walker, B.M. and Ettenberg, A. (2001) Intracerebroventricular ethanol potentiates the rewarding properties of intravenous heroin. *Society for Neuroscience Abstracts*

370. Walker, B.M. and Ettenberg, A. (2002) Intra-accumbens dopamine blockade attenuates the development of conditioned place preferences induced by centrally administered ethanol. *Society for Neuroscience Abstracts*

371. Weeks J.R. (1962) Experimental morphine addiction: Method for automatic intravenous injections in unrestrained rats. *Science 138*: 143-144

372. Weeks J.R., Collins R.J. (1978) Self-administration of morphine in the rat: relative influence of fixed ratio and time-out. *Pharmacology Biochemistry and Behavior 9*: 703-704

373. Wenger G.R. (1986) Behavioral effects of the isomers of pentobarbital and secobarbital in mice and rats. *Pharmacology Biochemistry and Behavior 25*: 375-380

374. Wesson D.R., Smith D.E. (1977) *Barbiturates: Their use, misuse, and abuse.* Human Sciences Press, New York

375. White N.M., Carr G.D. (1985) The conditioned place preference is affected by two independent reinforcement processes. *Pharmacology Biochemistry and Behavior 23*: 37-42

376. Wikler A. (1968) Diagnosis and treatment of drug dependence of the barbiturate type. *American Journal of Psychiatry 125*: 758-765

377. Wilks L.J., File S.E. (1988) Evidence for simultaneous anxiolytic and aversive effects several hours after administration of sodium phenobarbitone to the rat. *Neuropsychobiology 19*: 86-89

378. Williams K.L., Winger G., Pakarinen E.D., Woods J.H. (1998) Naltrexone reduces ethanol- and sucrose-reinforced responding in rhesus monkeys. *Psychopharmacology 139*: 53-61

379. Winger G., Herling S. (1982) Discriminative stimulus effects of pentobarbital in rhesus monkeys: tests of stimulus generalization and duration of action. *Psychopharmacology 76*: 172-176

380. Winger G., Stitzer M.L., Woods J.H. (1975) Barbiturate-reinforced responding in rhesus monkeys: comparisons of drugs with different durations of action. *Journal of Pharmacology and Experimental Therapeutics 195*: 505-514

381. Winger G.D., Woods J.H. (1973) The reinforcing property of ethanol in the rhesus monkey. I. Initiation, maintenance and termination of intravenous ethanol- reinforced responding. *Annals of the New York Academy of Sciences 215*: 162-165

382. Wirtshafter D., Klitenick M.A. (1989) Comparative studies of locomotor behavior following microinjections of muscimol into various sites in the paramedian

tegmentum. *Pharmacology Biochemistry and Behavior 32*: 625-628

383. Wise R.A. (1987) The role of reward pathways in the development of drug dependence. *Pharmacology and Therapeutics 35*: 227-263

384. Wise R.A. (1989) Opiate reward: sites and substrates. *Neuroscience and Biobehavioral Reviews 13*: 129-133

385. Wise R.A., Bozarth M.A. (1987) A psychomotor stimulant theory of addiction. *Psychological Review 94*: 469-492

386. Wise R.A., Leone P., Rivest R., Leeb K. (1995) Elevations of nucleus accumbens dopamine and DOPAC levels during intravenous heroin self-administration. *Synapse 21*: 140-148

387. Wise R.A., Yokel R.A., de Wit H. (1976) Both positive reinforcement and conditioned aversion from amphetamine and from apomorphine in rats. *Science 191*: 1273-1275

388. Wong D.T., Threlkeld P.G., Bymaster F.P., Squires R.F. (1984) Saturable binding of 35S-t-butylbicyclophosphorothionate to the sites linked to the GABA receptor and the interaction with GABAergic agents. *Life Sciences 34*: 853-860

389. Wong E.H., Snowman A.M., Leeb-Lundberg L.M., Olsen R.W. (1984) Barbiturates allosterically inhibit GABA antagonist and benzodiazepine inverse agonist binding. *European Journal of Pharmacology 102*: 205-212

390. Woods J.H., Katz J.L., Winger G. (1987) Abuse liability of benzodiazepines. *Pharmacological Reviews 39*: 251-413

391. World Health Organization (2001) *Global status report on alcohol.*

392. Xi Z.X., Stein E.A. (1998) Nucleus accumbens dopamine release modulation by mesolimbic GABAA receptors-an in vivo electrochemical study. *Brain Research 798*: 156-165

393. Xi Z.X., Stein E.A. (2000) Increased mesolimbic GABA concentration blocks heroin self-administration in the rat. *Journal of Pharmacology and Experimental Therapeutics 294*: 613-619

394. Yaksh T.L., Rudy T.A. (1978) Narcotic analgestics: CNS sites and mechanisms of action as revealed by intracerebral injection techniques. *Pain 4*: 299-359

395. Yaksh T.L., Yeung J.C., Rudy T.A. (1976) Systematic examination in the rat of brain sites sensitive to the direct application of morphine: observation of differential effects within the periaqueductal gray. *Brain Research 114*: 83-103

396. Yanagita T., Takahashi S. (1970) Development of tolerance to and physical dependence on barbiturates in rhesus monkeys. *Journal of Pharmacology and Experimental Therapeutics 172*: 163-169

397. Yokel R.A., Wise R.A. (1975) Increased lever pressing for amphetamine after pimozide in rats: implications for a dopamine theory of reward. *Science 187*: 547-

549

398. Yokel R.A., Wise R.A. (1976) Attenuation of intravenous amphetamine reinforcement by central dopamine blockade in rats. *Psychopharmacology 48*: 311-318

399. Yokel R.A., Wise R.A. (1978) Amphetamine- type reinforcement by dopaminergic agonists in the rat. *Psychopharmacology 58*: 289-296

400. York J.L. (1978) A comparison of the discriminative stimulus effects of ethanol, barbital, and phenobarbital in rats. *Psychopharmacology 60*: 19-23

401. York J.L., Bush R. (1982) Studies on the discriminative stimulus properties of ethanol in squirrel monkeys. *Psychopharmacology 77*: 212-216

402. Yu S., Ho I.K. (1990) Effects of GABA antagonists, SR 95531 and bicuculline, on GABAA receptor-regulated chloride flux in rat cortical synaptoneurosomes. *Neurochemical Research 15*: 905-910

403. Zahm D.S., Heimer L. (1993) Specificity in the efferent projections of the nucleus accumbens in the rat: comparison of the rostral pole projection patterns with those of the core and shell. *Journal of Comparative Neurology 327*: 220-232

404. Zangen A., Ikemoto S., Zadina J.E., Wise R.A. (2002) Rewarding and psychomotor stimulant effects of endomorphin-1: anteroposterior differences within the ventral tegmental area and lack of effect in nucleus accumbens. *Journal of Neuroscience 22*:

7225-7333

405. Zarevics P., Setler P.E. (1979) Simultaneous rate-independent and rate-dependent assessment of intracranial self-stimulation: evidence for the direct involvement of dopamine in brain reinforcement mechanisms. *Brain Research 169*: 499-512

406. Zimmerberg B., Brett M.B. (1992) Effects of early environmental experience on self-administration of amphetamine and barbital. *Psychopharmacology 106*: 474-478

407. Zito K.A., Vickers G., Roberts D.C. (1985) Disruption of cocaine and heroin self-administration following kainic acid lesions of the nucleus accumbens. *Pharmacology Biochemistry and Behavior 23*: 1029-1036

Appendix 1 - Time Spent

Experiment	Thesis Section	Dose X Compartment Interaction	Dose Main Effect	Compartment Main Effect
PENT CPP	2.2.	$F_{(3,28)} = 0.98$, NS	$F_{(3,28)} = 0.80$, NS	$F_{(1,28)} = 6.58$, $p < 0.025$
Bicuculline + PENT CPP	3.2.	$F_{(3,28)} = 1.56$, NS	$F_{(3,28)} = 1.04$, NS	$F_{(1,28)} = 13.38$, $p < 0.01$
Picrotoxin + PENT CPP	3.2.	$F_{(3,28)} = 0.06$, NS	$F_{(3,28)} = 1.40$, NS	$F_{(1,28)} = 24.47$, $p < 0.01$
Eticlopride + Amphetamine CPP	3.2.	$F_{(3,28)} = 3.41$, $p < 0.05$	$F_{(3,28)} = 1.47$, NS	$F_{(1,28)} = 26.53$, $p < 0.01$
Eticlopride + PENT CPP	3.2.	$F_{(3,28)} = 2.676$, $p = 0.066$	$F_{(3,28)} = 0.73$, NS	$F_{(1,28)} = 11.138$, $p < 0.01$
Naloxone + PENT CPP	3.2.	$F_{(3,28)} = 0.98$, NS	$F_{(3,28)} = 2.24$, NS	$F_{(1,28)} = 0.61$, NS
Naloxone + Vehicle CPP	3.2.	$F_{(2,21)} = 0.41$, NS	$F_{(2,21)} = 4.65$, $p < 0.05$	$F_{(1,21)} = 0.22$, NS
Systemic Barbital CPP	4.2.	$F_{(4,55)} = 2.58$, $p < 0.05$	$F_{(4,55)} = 0.16$, NS	$F_{(1,55)} = 0.66$, NS
ICV Barbital CPP	4.2.	$F_{(4,51)} = 2.47$, $p = 0.056$	$F_{(4,51)} = 2.12$, NS	$F_{(1,51)} = 3.04$, NS
Intra-PAG CPP	5.2.1.	$F_{(4,45)} = 1.57$, NS	$F_{(4,45)} = 0.17$, NS	$F_{(1,45)} = 1.89$, NS

Experiment	Thesis Section	Dose X Compartment Interaction	Dose Main Effect	Compartment Main Effect
Intra-pVTA CPP	5.2.1.	$F_{(3,36)} = 3.07$, $p < 0.05$	$F_{(3,36)} = 4.04$, $p < 0.025$	$F_{(1,36)} = 3.02$, NS
Intra-PH CPP	5.2.1.	$F_{(2,21)} = 0.97$, NS	$F_{(2,21)} = 1.21$, NS	$F_{(1,21)} = 0.11$, NS
Intra-LA CPP	5.2.1.	$F_{(2,22)} = 0.20$, NS	$F_{(2,22)} = 0.13$, NS	$F_{(1,22)} = 0.34$, NS
Intra-CeA CPP	5.2.1.	$F_{(3,34)} = 0.72$, NS	$F_{(3,34)} = 1.56$, NS	$F_{(1,34)} = 0.20$, NS
Intra-aVTA CPP	5.2.1.	$F_{(2,23)} = 0.10$, NS	$F_{(2,23)} = 0.07$, NS	$F_{(1,23)} = 0.18$, NS
Intra-PAG NMI + ICV barbital CPP	6.2.	$F_{(3,25)} = 1.79$, NS	$F_{(3,25)} = 1.29$, NS	$F_{(1,25)} = 3.68$, NS
Intra-pVTA NMI + ICV barbital CPP	6.2.	$F_{(2,21)} = 1.76$, NS	$F_{(2,21)} = 0.27$, NS	$F_{(1,21)} = 5.63$, $p < 0.05$
Intra-PAG SR 95531 + ICV barbital CPP	6.2.	$F_{(3,30)} = 0.97$, NS	$F_{(3,30)} = 1.12$, NS	$F_{(1,30)} = 5.41$, $p < 0.05$
Intra-pVTA SR 95531 + ICV barbital CPP	6.2.	$F_{(2,24)} = 0.67$, NS	$F_{(2,24)} = 0.16$, NS	$F_{(1,24)} = 7.55$, $p < 0.025$

Abbreviations are: aVTA = anterior ventral tegmental area; CeA = central amygdala; CPP = conditioned place preference; ICV = intracerebroventricular; LA = lateral amygdala; NMI = naloxone methiodide; PAG = periaqueductal gray; PENT = pentobarbital; PH = posterior hypothalamus; pVTA = posterior ventral tegmental area

Appendix 2 - Locomotor Activity

Experiment	Thesis Section	Dose X Compartment Interaction	Dose Main Effect	Compartment Main Effect
PENT CPP	2.2.	$F_{(3,28)} = 0.34$, NS	$F_{(3,28)} = 1.00$, NS	$F_{(1,28)} = 4.70$, $p < 0.05$
Bicuculline + PENT CPP	3.2.	$F_{(3,28)} = 0.12$, NS	$F_{(3,28)} = 0.77$, NS	$F_{(1,28)} = 4.97$, $p < 0.05$
Picrotoxin + PENT CPP	3.2.	$F_{(3,28)} = 0.08$, NS	$F_{(3,28)} = 0.38$, NS	$F_{(1,28)} = 16.88$, $p < 0.01$
Eticlopride + Amphetamine CPP	3.2.	$F_{(3,28)} = 1.77$, NS	$F_{(3,28)} = 2.63$, NS	$F_{(1,28)} = 13.68$, $p < 0.01$
Eticlopride + PENT CPP	3.2.	$F_{(3,28)} = 0.49$, NS	$F_{(3,28)} = 0.29$, NS	$F_{(1,28)} = 10.43$, $p < 0.01$
Naloxone + PENT CPP	3.2.	$F_{(3,28)} = 0.25$, NS	$F_{(3,28)} = 1.50$, NS	$F_{(1,28)} = 0.34$, NS
Naloxone + Vehicle CPP	3.2.	$F_{(2,21)} = 0.72$, NS	$F_{(2,21)} = 3.72$, $p < 0.05$	$F_{(1,21)} = 0.25$, NS
Systemic Barbital CPP	4.2.	$F_{(4,55)} = 0.75$, NS	$F_{(4,55)} = 0.76$, NS	$F_{(1,55)} = 0.28$, NS
ICV Barbital CPP	4.2.	$F_{(4,51)} = 1.60$, NS	$F_{(4,51)} = 0.75$, NS	$F_{(1,51)} = 0.64$, NS
Intra-PAG CPP	5.2.1.	$F_{(4,45)} = 1.42$, NS	$F_{(4,45)} = 1.15$, NS	$F_{(1,45)} = 0.05$, NS

Experiment	Thesis Section	Dose X Compartment Interaction	Dose Main Effect	Compartment Main Effect
Intra-pVTA CPP	5.2.1.	$F_{(3,36)} = 0.82$, NS	$F_{(3,36)} = 1.14$, NS	$F_{(1,36)} = 2.69$, NS
Intra-PH CPP	5.2.1.	$F_{(2,21)} = 0.54$, NS	$F_{(2,21)} = 0.14$, NS	$F_{(1,21)} = 0.23$, NS
Intra-LA CPP	5.2.1.	$F_{(2,22)} = 0.23$, NS	$F_{(2,22)} = 1.23$, NS	$F_{(1,22)} = 0.36$, NS
Intra-CeA CPP	5.2.1.	$F_{(3,34)} = 0.82$, NS	$F_{(3,34)} = 2.34$, NS	$F_{(1,34)} = 2.70$, NS
Intra-aVTA CPP	5.2.1.	$F_{(2,23)} = 1.47$, NS	$F_{(2,23)} = 0.18$, NS	$F_{(1,23)} = 0.47$, NS
Intra-PAG NMI + ICV barbital CPP	6.2.	$F_{(3,25)} = 1.37$, NS	$F_{(3,25)} = 0.46$, NS	$F_{(1,25)} = 4.35$, $p < 0.05$
Intra-pVTA NMI + ICV barbital CPP	6.2.	$F_{(2,21)} = 0.86$, NS	$F_{(2,21)} = 3.56$, NS	$F_{(1,21)} = 1.30$, NS
Intra-PAG SR 95531 + ICV barbital CPP	6.2.	$F_{(3,30)} = 0.56$, NS	$F_{(3,30)} = 0.70$, NS	$F_{(1,30)} = 4.09$, $p = 0.052$
Intra-pVTA SR 95531 + ICV barbital CPP	6.2.	$F_{(2,24)} = 2.27$, NS	$F_{(2,24)} = 0.39$, NS	$F_{(1,24)} = 1.66$, NS

Abbreviations are: aVTA = anterior ventral tegmental area; CeA = central amygdala; CPP = conditioned place preference; ICV = intracerebroventricular; LA = lateral amygdala; NMI = naloxone methiodide; PAG = periaqueductal gray; PENT = pentobarbital; PH = posterior hypothalamus; pVTA = posterior ventral tegmental area