School of Medicine
Oregon Health & Science University

CERTIFICATE OF APPROVAL

This is to certify that the Ph.D. thesis of

Paul J. Meyer

has been approved

[redacted]
Tamara J. Phillips, Mentor

[redacted]
Charles K. Meshul, Committee Chair

[redacted]
William R. Woodward, Committee Member

[redacted]
Robert J. Hitzemann, Committee Member

[redacted]
Gregory P. Mark, Committee Member

Neurochemical Substrates of Ethanol's Locomotor Effects

A Dissertation by Paul J. Meyer

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To my dad Jerry and wife Rebecca, for teaching me all the important things.

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Abbreviations

5-HT – 5-hydroxytryptamine (serotonin)
6-OHDA – 6-hydroxydopamine
AA – Alko alcohol (a selected rat line)
ANA – Alko non-alcohol (a selected rat line)
aCSF – artificial cerebrospinal fluid
ACT score – activity score (response to ethanol minus response to saline)
ANOVA – analysis of variance
BEC – blood ethanol concentration
cAMP – cyclic adenosine monophosphate
CeA – central nucleus of the amygdala
DAT – dopamine transporter
DARPP-32 – dopamine receptor phosphoprotein, 32 kiloDaltons
fos-li – fos-like immunoreactivity
fMRI – functional magnetic resonance imaging
GABA – γ-amino butyric acid
HAD – high alcohol drinking (a selected mouse line)
HAS – high alcohol sensitivity (a selected rat line)
HPLC – high performance liquid chromatography
ICSS – intracranial self-stimulation
i.p. – intraperitoneal
LAD – low alcohol drinking (a selected mouse line)
LAS – low alcohol sensitivity (a selected rat line)
LS – long-sleep (a selected mouse line)
NAcc – nucleus accumbens
NMDA – N-methyl-D-aspartate
NP – alcohol nonpreferring (a selected rat line)
P – alcohol preferring (a selected rat line)
PBS – phosphate-buffered saline
PCP – phencyclidine
PE – polyethylene
PET – positron emission tomography
PFC – prefrontal cortex
SEM – standard error of the mean
SN – substantia nigra
SS – short-sleep (a selected mouse line)
TH – tyrosine hydroxylase
VP – ventral pallidum
VTA – ventral tegmental area

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Abstract

Neuroanatomical research has revealed that the mesolimbic dopamine system is involved in both drug-induced locomotion and addiction. Neurons projecting from the ventral tegmental area (VTA) to forebrain areas including the nucleus accumbens (NAcc) and central nucleus of the amygdala (CeA) may be particularly important for the locomotor response to ethanol. To study this, we conducted electrolytic lesions of the VTA, NAcc, and CeA in mice selectively bred for high (FAST) and low (SLOW) sensitivity to ethanol's locomotor stimulant effects. In addition, we measured drug-induced changes in NAcc dopamine and glutamate using *in-vivo* microdialysis. Lesions of the VTA attenuated the locomotor stimulant response to ethanol in FAST mice, but lesions of the NAcc and CeA had no effect. The sedative response to ethanol in SLOW mice was not affected by any lesion. In microdialysis studies, ethanol and cocaine resulted in increases in dopamine within the NAcc, but to a greater degree in FAST mice. There was no effect of either drug on NAcc glutamate levels. In a final study, VTA lesions attenuated the ethanol-induced increases in NAcc dopamine in FAST mice. These experiments indicate that 1) the mesolimbic dopamine system modulates the locomotor stimulant response to ethanol and 2) changes in dopamine systems within the nucleus accumbens are genetically correlated with ethanol- and cocaine- induced locomotion.

Introduction

Drug addiction is a pervasive disease and a grievous problem for both the individual and for society (Rice, 1999; Volpicelli, 2001). Alcohol (ethanol) abuse and alcoholism affect approximately 14 million Americans, and the management of alcoholism and its associated health problems costs society approximately $185 billion per year in terms of health care, reduced productivity, and legal costs (Grant et al., 2001; Harwood, 2000; McGinnis and Foege, 1999). For these reasons, experimental research on the factors and processes that lead to the development of addiction in humans and in animal models is important for developing treatment and prevention strategies. Human studies, because of the prevalence of alcoholism amongst genetically related individuals, can be utilized to study the genetic nature of alcoholism (Schuckit et al., 2004). Humans can also give subjective reports on the affective properties of ethanol (Chutuape and de Wit, 1994), which is advantageous compared to making inferences from indirect measures in animal studies. Animal models of ethanol addiction allow superior experimental control compared to human studies, and permit the use of more invasive techniques (Stewart and Li, 1997). Combined results from translational human and animal research have been successful in describing some of the neurobiological processes that confer sensitivity to ethanol and its addictive properties. In turn, these properties may bear a relationship to the risk for development of alcoholism.

Modeling ethanol's effects experimentally

The complex, multidimensional nature of alcoholism makes it difficult to model experimentally. Therefore, most models focus on a particular feature of alcoholism.

Human and animal studies of ethanol sensitivity involve the initial response to various acute effects of ethanol, such as euphoria and sedation, and some attempt to relate this sensitivity to the initiation and maintenance of sustained drinking (Da Silva et al., 2005). Other models focus on the neuroadaptive effects of chronic ethanol drinking or exposure, such as withdrawal, tolerance, and sensitization (Becker and Lopez, 2004; Kalant et al., 1971; Lopez and Becker, 2005; Phillips et al., 1995). More recently, research involving relapse into excessive drinking after a period of abstinence has suggested that the neural mechanisms of relapse are distinct from those regulating the initiation and maintenance of drinking (Weiss and Porrino, 2002).

Of particular interest is that some individuals seem more likely to engage in excessive drinking than others; a phenomenon that applies to both animal and human subjects (Crabbe et al., 1992; Heath et al., 1999). Most researchers assume that ethanol drinking begins because ethanol has rewarding or reinforcing properties that encourage further ingestion of ethanol (Gonzales et al., 2004; Koob et al., 2004; Samson and Czachowski, 2003). Therefore, paradigms that examine the reinforcing properties of ethanol are particularly useful in studying the sources of individual variation in ethanol drinking (Cunningham et al., 2000; Rhodes et al., 2005). Self administration, in which an animal drinks freely from a supply of ethanol (Richter and Campbell, 1940) or performs a simple task such as pressing a lever for a presentation of ethanol (McMillan and Leander, 1978), is a commonly used approach. The findings that certain animals will prefer ethanol to water (McClearn and Rodgers, 1959) or perform several lever presses to obtain ethanol (Roehrs and Samson, 1981) suggest that ethanol is a reinforcer and has rewarding properties.

Conditioned place preference is another commonly used paradigm that utilizes Pavlovian conditioning techniques to measure ethanol reinforcement by administering ethanol to an animal within a particular environment and then measuring the animal's relative preference for that environment (Cunningham, 1995; Cunningham et al., 2000). Research suggests that the ethanol-associated environment can serve as a reinforcer, depending on the type of animal or the specific experimental procedure used (Ciccocioppo et al., 1999; Cunningham et al., 2002; Cunningham et al., 1992b). It is likely that this measures a subtype of reward that is dissociable from that measure in self-administration paradigms, because significant genetic correlations have not been measured between ethanol drinking and ethanol-induced conditioned place preference in BXD recombinant inbred mice (Phillips et al., 1998).

Another conditioning approach used to study ethanol reinforcement is conditioned taste aversion, in which a taste stimulus is paired with ethanol administration. This pairing results in the subsequent avoidance of the ethanol-paired solution (Berman and Cannon, 1974; Linakis and Cunningham, 1979; Nachman et al., 1970). Similar findings have been found for other abused drugs (Cappell et al., 1973; D'Mello et al., 1977; Goudie et al., 1978). The apparent paradox of a presumably reinforcing drug stimulus resulting in a conditioned taste aversion has been suggested to be related to the novelty of the drug state, which researchers have referred to as "drug shyness" (Hunt and Amit, 1987) and "taste avoidance" (Parker, 1995), rather than taste aversion. However, significant *negative* genetic correlations among conditioned taste aversion and ethanol drinking have been found using a panel of inbred mouse strains (Broadbent et al., 2002). In other words, mice that were more sensitive to ethanol-induced conditioned taste

aversion drank less ethanol, a finding that does not support conditioned taste aversion as a measure of reward. However, this negative correlation was not found in BXD recombinant inbred mice (Risinger and Cunningham, 1998).

While most studies use self-administration or conditioning to assess reinforcement and reward, other paradigms have also been used. For example, in an intra-cranial self-stimulation (ICSS) study, an animal's reward pathway is directly stimulated via an intra-cerebral electrode as it presses an appropriate lever in a self-administration chamber. Reinforcing stimuli, such as drugs of abuse, tend to decrease the threshold current required to maintain the self-administration behavior, while aversive stimuli tend to increase this threshold (Cassens and Mills, 1973; Olds and Milner, 1954; Schaefer and Holtzman, 1979). Ethanol will also decrease this threshold if it is voluntarily ingested (Bain and Kornetsky, 1989) but not if injected (Schaefer and Michael, 1987), suggesting that the route of administration is an important variable in determining whether ethanol is reinforcing within a particular paradigm. ICSS has been used to study drug reinforcement (Bossert and Franklin, 2003; Mague et al., 2005; Todtenkopf et al., 2004) and to asses various hedonic states, such as those induced by drug withdrawal (Barr et al., 2002) and drug-associated cues (Hayes and Gardner, 2004).

These models are useful tools for studying ethanol reinforcement in that they incorporate multiple psychological and biological processes thought to underlie the development of alcohol addiction (Samson and Czachowski, 2003; Stewart et al., 1988). However, these same processes complicate the interpretation of certain findings obtained with these paradigms. For example, these models all require some form of learning or conditioning (Bienkowski et al., 1999). Furthermore, experimental and pharmacological

manipulations that alter performance on these tasks may have their effect by interfering with learning processes (Khanna et al., 1994). A particular mouse strain may display preference for an ethanol-associated environment because it is a better learner than other strains, and not necessarily because it finds ethanol more rewarding. Interpretational problems are also common when pharmacological manipulations that are effective at decreasing ethanol drinking or self-administration have sedative effects that result in non-specific effects on behaviors such as locomotion (Escher and Mittleman, 2004), drinking (Silvestre et al., 1996), and anhedonia (de Wit et al., 1999). Taste factors and peripheral actions of ethanol have been suggested to play a role in ethanol drinking paradigms (Belknap et al., 1977), although most researchers agree that ethanol-induced conditioned taste aversion is not due to its actions on central systems (Eckardt, 1975; Sklar and Amit, 1977).

The relationship between ethanol stimulation and addiction

Limited availability of experimental control in human studies and interpretational problems encountered during self-administration and conditioning studies make the neurobiological determinants of ethanol's rewarding and reinforcing effects difficult to study. However, while the rewarding effect of an acute administration of ethanol is difficult to measure, ethanol has several acute effects easily measured in humans and animals. Some important examples of ethanol's acute behavioral effects are ataxia (Crabbe, 1983; Schuckit, 1985), hypnosis (loss of righting reflex) (Baker et al., 1987; Crabbe, 1983; Sanders et al., 1978), anti-convulsion (Newland and Weiss, 1991; Rajput et al., 1975), anxiolysis (Lister and File, 1983; Stinchcomb et al., 1989), hypothermia (Crabbe, 1983; Kalant and Le, 1983), psychomotor stimulation (Davidson et al., 2002;

Dudek and Phillips, 1983), and sedation (Sanders and Sharpless, 1978; Zacny et al., 1994). The role of these various acute effects in the development of excessive ethanol drinking and addiction is an actively studied area of research. In fact, some researchers have suggested that the initial response to ethanol may predict an individual's tendency to develop an addiction to the drug (Heath et al., 2001; Holdstock et al., 2000; Kalant and Le, 1983; Newlin and Thomson, 1991; Schuckit, 1994; Schuckit and Smith, 2001).

Human Studies

The initial sensitivity to the stimulant and sedative effects of ethanol has received particular attention. There is evidence from both human and animal studies that suggests the sensitivity to ethanol's stimulant effects, as well as insensitivity to its sedative effects, is positively associated with propensity to self-administer ethanol. For example, studies by Shuckit and colleagues have found that sons of alcoholics were less sensitive to different measures of ethanol intoxication, including subjective sedation (Schuckit, 1980), motor incoordination (Schuckit, 1985), and ethanol-induced alterations of EEG recordings (Schuckit et al., 1988). However, other studies have found that heavy drinkers were more sensitive to subjective ethanol stimulation than their light-drinking counterparts, as measured by self-report questionnaires such as the Profile of Mood States and the Addiction Research Center Inventory (de Wit et al., 1987; Duka et al., 1998). These apparently discrepant findings may be due to ethanol's biphasic actions. Both ethanol-induced stimulation and sedation often occur after a single administration of ethanol (Pohorecky, 1977), with stimulation occurring during the initial increase in the blood ethanol levels, and sedation occurring during the subsequent decline (Holdstock and de Wit, 1998). Studies using social drinkers have suggested that subjects with

heavier drinking patterns are more sensitive to the stimulant effects of ethanol, but less sensitive to its sedative effects, compared to those who only drank occasionally (Holdstock et al., 2000; King et al., 2002). However, it is unclear if these differences in sensitivity were pre-existing traits or a result of the heavy drinkers' excessive drinking history. One way to approach this problem is by studying subjects with a family history of alcoholism who have had limited experience with ethanol. Newlin and Thomson (1991) investigated sons of alcoholics and found that ethanol-induced stimulation was greater in subjects with a family history of alcoholism. This raises the possibility that sensitivity to ethanol-induced stimulation, and insensitivity to ethanol-induced sedation, may play an important role in the development of alcoholism.

Animal Studies

In rodents, a common effect of many drugs of abuse is their ability to stimulate locomotor behavior (Amalric and Koob, 1993; Phillips et al., 1992; Tzschentke and Schmidt, 2000). This has led some researchers to suggest that the locomotor and reinforcing effects of a drug are the result of activation of a common neurobiological substrate (Wise and Bozarth, 1987). However, support for this theory is mixed. In the case of ethanol, several experimental studies have dissociated these drug effects. Sprague-Dawley and Wistar rats, which can be trained to self-administer ethanol to the point of physiological dependence, typically show locomotor sedation, rather than stimulation, in response to an ethanol injection (Erickson and Kochhar, 1985). Also, while DBA/2J mice show robust stimulant responses to ethanol and C57BL/6J mice do not, DBA/2J mice typically will not drink an ethanol solution, while C57BL/6J will drink readily (Phillips, 1993). However, preabsorptive factors such as taste and smell have

been found to influence ethanol consumption in these strains (Belknap et al., 1993; McMillen and Williams, 1998; Phillips et al., 1994). Using the conditioned place preference paradigm, which bypasses these taste factors, Cunnigham et al. (1992b) found that DBA/2J mice displayed more ethanol-induced locomotor stimulation and conditioned place preference than C57BL/6J mice. However, in a study of 20 recombinant inbred stains generated from an intercross of C57BL/6J and DBA/2J mice, no genetic correlation between ethanol-induced stimulation and conditioned place preference was found (Cunningham, 1995). Pharmacological support for this theory is also mixed. Haloperidol, a non-specific dopamine receptor antagonist, blocks the stimulant and reinforcing effects of ethanol in humans (Enggasser and de Wit, 2001) and rodents (Pfeffer and Samson, 1988; Risinger et al., 1992). However, ethanol-induced conditioned place preference was not affected by this drug (Cunningham et al., 1992a; Risinger et al., 1992). Another example is baclofen, an agonist of the γ-amino-butyric acid ($GABA_B$) receptor, which has been shown to block the locomotor response to ethanol (Shen et al., 1998) and drinking (Colombo et al., 2004; Daoust et al., 1987), but not ethanol-induced conditioned place preference or taste aversion (Chester and Cunningham, 1999).

Studies with selected lines

As in humans, sensitivity to the stimulant and sedative effects of ethanol in animals is variable among individuals (Phillips et al., 1995). Beginning with a heterogenous population of mice created by a cross of eight inbred strains, our laboratory has used selective breeding techniques to derive mice with high (FAST) and low (SLOW) locomotor stimulant responses to ethanol (Crabbe et al., 1987; Phillips et al.,

1991). The response to selection over the first 37 generations of selection is shown in figure 1. SLOW mice are not only resistant to ethanol stimulation but also more sensitive to ethanol's locomotor sedative effects.

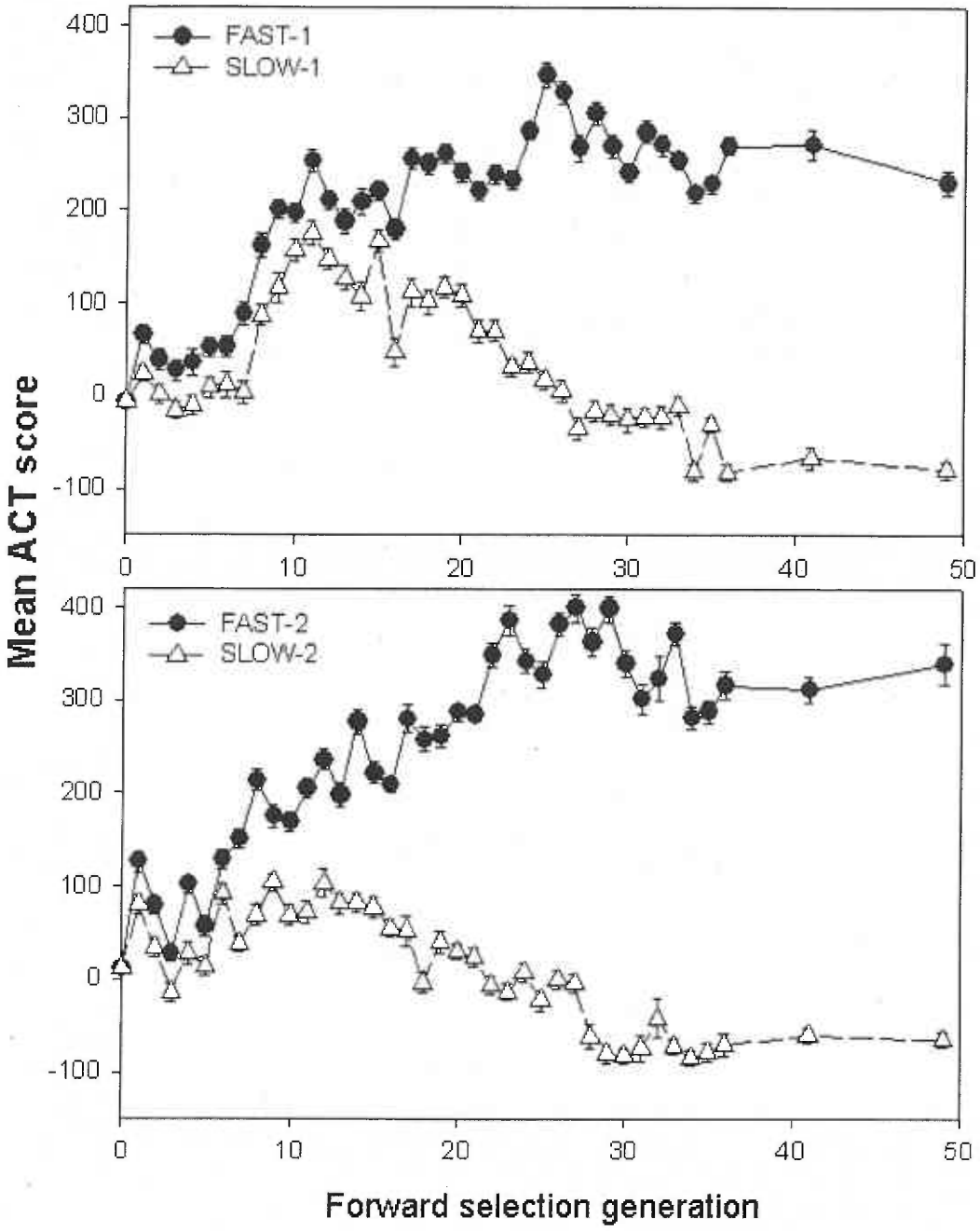


Figure 1: Response to selection for increased (FAST) and decreased (SLOW) locomotor activity in response to 2 g/kg ethanol. "ACT score" refers to ethanol-induced activity minus saline-induced activity measured on a separate day. The top and bottom panel depicts the responses in replicates 1 and 2, respectively.

Selected lines such as these are useful in determining traits that may be genetically correlated with the selection trait. For example, hypothesizing that sensitivity to ethanol's locomotor effects is correlated with sensitivity to ethanol's reinforcing efficacy, Risinger et al. (1994) found that FAST mice drink larger amounts of ethanol than SLOW mice, although the lines did not differ in ethanol reinforcement, as measured by a place conditioning paradigm. This suggests that ethanol drinking and ethanol-induced locomotion may be related genetically, although other studies have not found this relationship (Sanchez et al., 1996). In studies with rats selectively bred for alcohol drinking, including alcohol preferring/non-preferring (P/NP) rats and high/low alcohol-drinking (HAD/LAD) rats, as well as those using Maudsely reactive/non-reactive rat lines, alcohol preference was positively correlated with ethanol's locomotor effects (Krimmer and Schechter, 1992; Li et al., 1987; Waller et al., 1986). In other words, selected lines that drank more also showed larger locomotor stimulant responses to ethanol, compared to their non-preferring counterparts. However, while consumed ethanol increased locomotion in another selectively bred, alcohol-preferring (AA) line of rats, systemic injections of ethanol revealed no differences in locomotor response compared to their ethanol-avoiding (ANA) counterparts (Paivarinta and Korpi, 1993). Also, Grahame et al., (2000) found no differences in ethanol-induced locomotion in mice bred for alcohol preference, although sensitization to the initial locomotor effect correlated with high levels of ethanol drinking. Finally, another selective breeding project produced mice with high (long-sleep or LS) and low (short-sleep or SS) sensitivity to ethanol's hypnotic (loss of righting reflex) effects. Both FAST and SS mice display greater levels of ethanol-induced locomotion, decreased sensitivity to ethanol's

hypnotic effects, and larger ethanol intake compared to their SLOW and LS counterparts (Church et al., 1979; Erwin and Jones, 1993; Risinger et al., 1994). However, in another set of mice bred for high and low ethanol drinking (Phillips et al., 2005), high drinking mice displayed greater ethanol-induced conditioned place preference, but either the same or *less* ethanol stimulation (depending on the test apparatus), compared to low drinking mice. These apparently discrepant responses may be due to the different starting populations used to produce FAST and SLOW (a cross of eight inbred strains) and high and low drinking mice (an F_2 intercross of DBA/2J and C57BL/6J). These results suggest that, at least in some mouse models of ethanol's effects, sensitivity to ethanol-induced locomotor stimulation is related to ethanol reinforcement.

The neurobiology of ethanol's locomotor effects

Ethanol has direct effects on several neurotransmitter receptors (i.e., it associates with certain receptors in the absence of interaction with other cellular components), as well as other cellular components such as ion channels. While no single receptor or other neural substrate is responsible for all of ethanol's effects on locomotion, the combination of these effects are probably responsible for ethanol-induced activation of brain systems that regulate locomotion. However, the neuroanatomical substrates of ethanol-induced stimulation have not been as extensively investigated, perhaps because the stimulant response to ethanol is not consistent across rat and mouse strains (Crabbe et al., 1994; Erickson and Kochhar, 1985). In the following section, ethanol's interactions with several neurotransmitter receptors and other membrane proteins are reviewed, and how these interactions may regulate ethanol-induced locomotion is discussed.

GABA receptors

Ethanol potently modulates subtypes of the GABA receptor. Specifically, ethanol acts allosterically at the $GABA_A$ channel to enhance the flux of chloride ions, resulting in neuronal inhibition (Allan et al., 1988). While the $GABA_B$ receptor has been less extensively studied, recent evidence suggests that ethanol enhances $GABA_B$ receptor-mediated effects, either directly or by potentiation of GABA release (Ariwodola and Weiner, 2004; Lewohl et al., 1999). Ethanol's GABAergic effects may be responsible for many of its behavioral effects (Boehm et al., 2004; Koob, 2004). $GABA_A$ receptor antagonists are effective at attenuating a variety of ethanol's acute effects (Grobin et al., 1998), including loss of righting reflex (Liljequist and Engel, 1982), motor incoordination (Martz et al., 1983), and anxiolysis (Becker and Hale, 1991). Genetic deletion of the alpha-1 subunit of the $GABA_A$ receptor enhanced the stimulant response to ethanol (Kralic et al., 2003), while $GABA_A$ receptor antagonists reduced the stimulant effects of ethanol (Chester and Cunningham, 1999; McKay et al., 2004). FAST mice were more sensitive to the stimulant effects of several GABAergic compounds, including diazepam, pentobarbital, and allopregnanolone (Palmer et al., 2002a; 2002b; 2002c), and less sensitive to the sedative effects of the $GABA_B$ receptor agonist, baclofen, in one replicate (Shen et al., 1998). Interestingly, baclofen also decreased the stimulant response to ethanol in FAST mice, perhaps due to its ability to inhibit dopamine-containing neurons in the ventral tegmental area (VTA) (Boehm et al., 2002a). However, the $GABA_A$ receptor antagonists picrotoxin and bicuculline had no effect on ethanol stimulation in FAST mice (Shen et al., 1998), suggesting that ethanol stimulation in FAST mice occurs independently of ethanol's actions at the $GABA_A$ receptor.

Other selected lines show similar correlations. The locomotor response to ethanol differs in rats selectively bred for high (HAS rats) and low (LAS rats) sensitivity to ethanol-induced hypnosis (loss of righting reflex), with HAS rats showing locomotor depression to 2 g/kg and LAS rats showing no locomotor response (Krimmer and Schechter, 1992). Ethanol potentiated chloride flux through $GABA_A$ receptors stimulated by the $GABA_A$ receptor agonist muscimol in membranes prepared from HAS mice, but not LAS mice, which suggests that differences in $GABA_A$ receptor sensitivity to ethanol may be genetically related to differences in behavioral sensitivity to ethanol (Allan et al., 1988). For example, LS mice, which are bred for enhanced sensitivity to ethanol-induced loss of righting reflex, were less sensitive to ethanol-induced stimulation relative to SS mice, and more sensitive to the hypnotic effects of various $GABA_A$ -acting barbiturates and benzodiazepines (McIntyre and Alpern, 1985). However, $GABA_A$ receptor antagonists reduced or potentiated ethanol-induced hypnosis in LS and SS mice, depending on the genotype and the specific antagonist used (Dudek and Phillips, 1983; Martz et al., 1983). This suggests that GABA receptors are involved in ethanol sensitivity, but this involvement is dependent on several pharmacological and genetic factors.

Glutamate receptors

Ethanol is an allosteric inhibitor of the N-methyl-D-aspartate (NMDA) subclass of glutamate receptors (Dildy and Leslie, 1989; Lovinger et al., 1989; Wright et al., 1996). Anesthetic doses of ethanol also inhibit the amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) subclass of glutamate receptors in the hippocampus (Wang et al., 1999). Ethanol inhibition of glutamatergic transmission may be an important

mediator of its stimulant properties, as non-competitive NMDA antagonists such as MK-801, phencyclidine (PCP), and ketamine have been shown to increase locomotion in rodents (Koek et al., 1989; Liljequist and Engel, 1982; Tricklebank et al., 1989). In selected mouse lines, sensitivity to ethanol's stimulant and hypnotic effect was genetically correlated with sensitivity to the NMDA antagonists MK-801 and ketamine (Hanania and Zahniser, 2002; Kuribara, 1994; Meyer and Phillips, 2003; Shen and Phillips, 1998). Meyer and Phillips (2003) further showed that, when given in combination, ethanol and ketamine had additive effects on locomotion, suggesting that these drugs have convergent effects on the neurochemical systems underlying locomotor stimulation. These convergent effects may be mediated by the NMDA receptor. Daniel and Phillips (1994) found that microsacs containing NMDA receptors from FAST and SLOW mice were differentially sensitive to ethanol's inhibitory effects. This difference in NMDA sensitivity to ethanol inhibition may be the reason that these mice were differentially sensitive to ethanol's stimulant effects.

Interestingly, non-competitive NMDA antagonists such as ethanol may elicit locomotion by actually enhancing glutamatergic transmission within the mesolimbic dopamine system, which includes dopamine projections from the VTA to the nucleus accumbens (NAcc; see next section for basic neuroanatomy). For example, Mathe et al. (1998) showed that non-NMDA antagonists administered directly into the VTA blocked the increases in NAcc dopamine as well as the increases in locomotion elicited by a systemic injection of MK-801. This suggests that MK-801 may enhance glutamatergic tone by inducing glutamate release, thereby resulting in the stimulation of cells in the VTA via non-NMDA receptors. The same may be true for ethanol. While Yan et al.

(1998) have shown that 2 g/kg ethanol caused a decrease in glutamate levels within the NAcc of Sprague-Dawley rats, this is a relatively high ethanol dose in rats, and this strain of rats typically show locomotor depression rather than activation in response to ethanol. Studies in HAS and LAS rats, which differ in sensitivity to the hypnotic and locomotor effects of ethanol, have found that HAS rats showed ethanol-induced decreases in glutamate levels within the NAcc while LAS rats showed an increase in glutamate levels (Dahchour et al., 2000). This suggests that ethanol induced locomotor activity, loss of righting reflex, and increases in NAcc glutamate may be genetically related. This further suggests that extracellular glutamate levels in the NAcc may increase in response to ethanol in FAST mice, and decrease in SLOW mice.

Nicotinic receptors

Ethanol has direct effects on other receptors and membrane proteins that are often overlooked. Ethanol has effects on the nicotinic subclass of acetylcholine receptors (El-Fakahany et al., 1983; Yu et al., 1996), which may participate in ethanol-induced stimulation and addiction (Bowers et al., 2005). Blomqvist et al. (1992) have shown that mecamylamine, a non-specific antagonist of nicotinic receptors, blocks the stimulant response to ethanol in DBA/2J and NMR1 mice. FAST and SLOW selected lines are differentially sensitive to nicotine's locomotor effects (Bergstrom et al., 2003), and the locomotor stimulant response to ethanol was blocked by mecamylamine in FAST mice (Kamens and Phillips, unpublished data). In mice with a genetic deletion of the $\alpha 7$ subunit of the nicotinic receptor, ethanol's stimulant and reinforcing effects were enhanced (Bowers et al., 2005), which also provides support for ethanol's locomotor stimulant and reinforcing effects having similar neural substrates.

Serotonin receptors

Ethanol has been shown to potentiate the effects of serotonin at 5-HT(3) serotonin receptors directly (Lovinger and White, 1991), which may tonically excite VTA neurons (Minabe et al., 1991; Rasmussen et al., 1991). Administration of 5-HT(3) antagonists blocked ethanol-induced increases in dopamine (Campbell and McBride, 1995) and reduced ethanol drinking (McKinzie et al., 1998), but the effects of these antagonists on ethanol stimulation has not been extensively studied. Genetic deletion of the 5-HT(3A) subtype of this receptor did not affect ethanol-induced locomotion, although there was no robust ethanol-induced stimulation in either the transgenic or wild-type mice in this study (Hodge et al., 2004). In mice bred for insensitivity to ethanol's hypnotic effects, ethanol-stimulated activity was blocked by 5-HT(2C) receptor antagonists but potentiated by 5-HT(1A) agonists. There is, however, no evidence that these effects are through a direct activity on these receptors.

Glycine Receptors

Glycine is a major inhibitory neurotransmitter in the mammalian central nervous system, and glycinergic transmission is important for the control of both motor and sensory functions in the spinal cord (Betz et al., 1999). Glycine has been shown to regulate locomotor-associated neuronal activity in the spinal cord of developing mice (Hinckley et al., 2005). Interestingly, glycine receptors within the NAcc regulate ethanol consumption in rats (Molander et al., 2005). While direct effects at the strychnine-sensitive glycine receptor have not been shown, injections of strychnine into the NAcc have been shown to modulate extracellular dopamine levels and ethanol consumption in

rats (Molander and Soderpalm, 2005a; 2005b). However, the role of glycine receptors in ethanol-induced locomotion has not been investigated.

Ion Channels

Ethanol also regulates membrane bound ion-channels including certain potassium and voltage gated calcium channels (Kobayashi et al., 1999; Lewohl et al., 1999; Messing et al., 1986). While the role of these channels in ethanol-induced locomotion is unclear, it is likely that they play an important role in ethanol's effects on behavior, given that many of these channels are widely expressed throughout the brain and have direct effects on neuronal excitability. In fact, it has been suggested that ethanol can promote activation of dopamine neurons through its inhibition of quinidine-sensitive potassium channels (Appel et al., 2003).

Ethanol's interactions with the mesolimbic dopamine system

In the cases of cocaine and amphetamine, experimental research has revealed substantial overlap in the brain areas that mediate both the locomotor stimulant and reinforcing effects of these drugs. Extensive studies of the mesolimbic dopamine system have indicated that this system is crucial in controlling motivated behavior and locomotion (Mogenson and Yang, 1991), as well as the activating and reinforcing properties of psychostimulants and opiate drugs (Amalric and Koob, 1993; Ikemoto and Panksepp, 1999; Kelly et al., 1975; Swerdlow et al., 1986; Tzschentke and Schmidt, 2000). Interestingly, there is no consistent evidence that ethanol acts directly upon dopamine receptors or dopamine transporters (Eshleman et al., 1994; Robinson et al., 2005; Yim and Gonzales, 2000). Further, there is mixed evidence for a genetic correlation between ethanol stimulation and dopamine D2 receptors (Bergstrom et al.,

2003; Hitzemann et al., 2003). However, ethanol likely influences dopaminergic signaling pathways through its actions at the receptor systems described above. For example, several ethanol-sensitive neurotransmitter receptors influence dopamine responsive proteins such as dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP-32) through the accumulation of cAMP, intracellular calcium, and protein kinase-A, as well as activation of protein phosphatases such as PP-2B. Through these interactions, dopamine systems may be an important mediator of ethanol's effects on physiology and behavior.

To support this, there is a growing body of evidence that ethanol activates the mesolimbic dopamine system (Gonzales et al., 2004; Imperato and Di Chiara, 1986; Phillips and Shen, 1996). Early evidence from pharmacological studies using dopamine antagonists showed that drugs such as haloperidol blocked the locomotor response to ethanol (Pfeffer and Samson, 1988; Risinger et al., 1992; Shen et al., 1995). Studies showing that dopamine antagonists blocked the stimulant response to ethanol in FAST mice (Shen et al., 1995) provide further evidence that dopaminergic systems are involved in ethanol stimulation (Phillips and Shen, 1996), and that the dopaminergic system may have been altered by selective breeding. While ethanol may regulate dopaminergic signaling through multiple mechanisms, some investigators have suggested that ethanol activates the dopamine system by blocking inhibitory input into dopaminergic brain areas, resulting in a disinhibition of dopaminergic neurons (Tzschentke and Schmidt, 2000). Others have argued that ethanol activates these neurons directly (Brodie et al., 1999). The following section discusses the basic neuroanatomy of the mesolimbic

dopamine system, common methods used to study its function, and how ethanol and other drugs of abuse may modulate its function.

Basic neuroanatomy

The basic neurocircuitry of the mesolimbic dopamine system is described in figure 2. The major outputs of this system are the GABAergic projections from the NAcc to the ventral pallidum (VP), which in turn project to the mediodorsal thalamus and on to motor output nuclei in the cortex. The NAcc-VP projection is interesting because increases in locomotion may be due to the inhibition of these projections (Mogenson et al., 1993; Pennartz et al., 1994). Glutamatergic inputs from the prefrontal cortex (PFC), amygdala, and the hippocampus, which provide excitatory input to NAcc neurons, are modulated by dopaminergic (and GABAergic) projections from the VTA to the NAcc (Kalivas et al., 1993). The VTA also indirectly modulates glutamate transmission in the NAcc through dopaminergic projections to the VP, PFC, amygdala, and hippocampus (Carr and Sesack, 2000a; Pirot et al., 1992; Oades and Halliday, 1987; Swanson, 1982; (Van Bockstaele and Pickel, 1995).

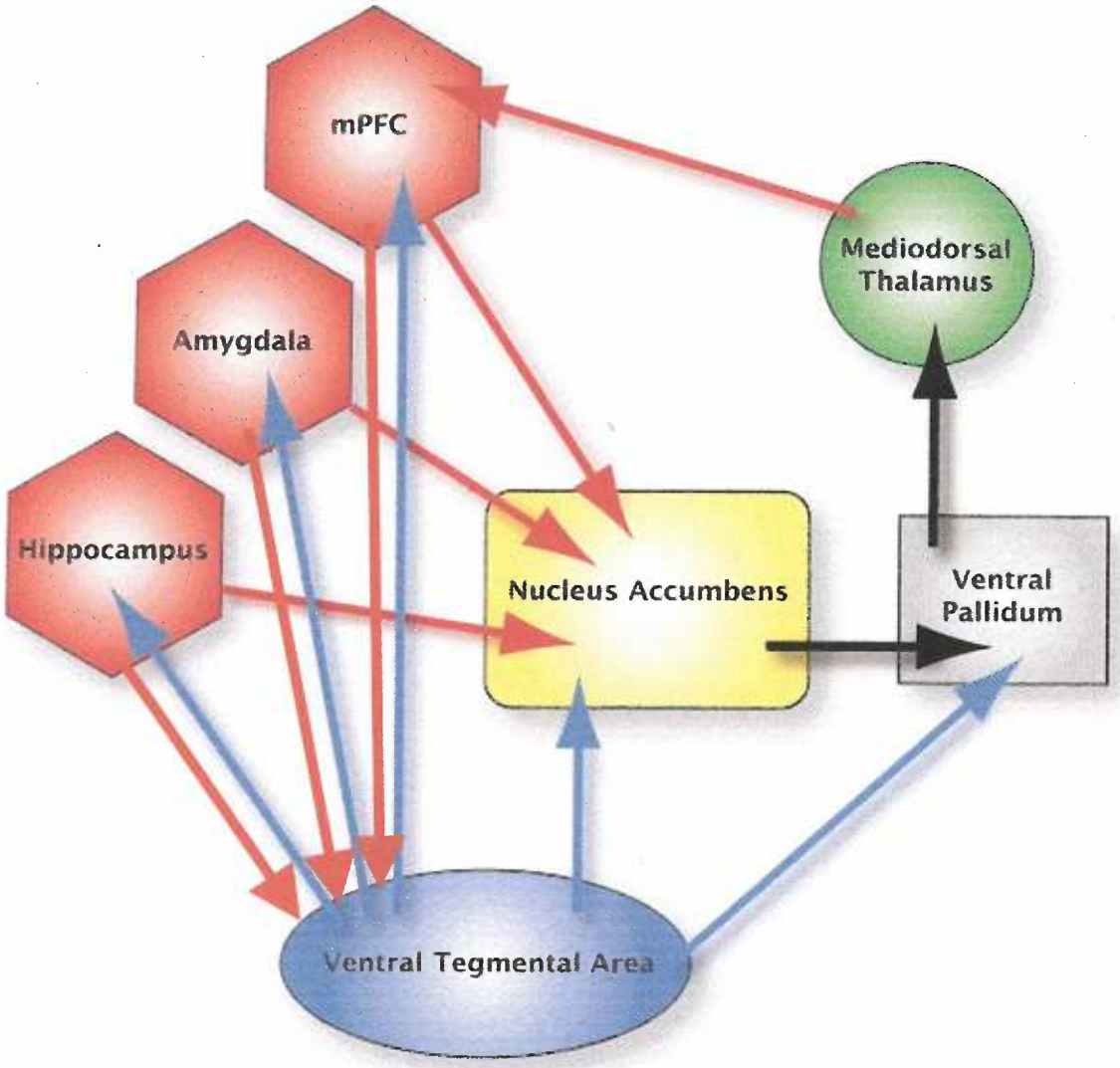


Figure 2: Mesolimbic circuitry. Red arrows indicate glutamatergic pathways; black arrows indicate GABAergic pathways; blue arrows indicate dopaminergic pathways. See text for additional details. Figure adapted from Pierce and Kumaresan (2005).

A number of feedback loops exist within this system. For example, NAcc neurons project back to the VTA (Churchill and Kalivas, 1994; Kalivas et al., 1993). Also of importance are reciprocal connections between the VTA and the amygdala (Fudge and Haber, 2000), and caudal projections from the VTA to the raphe nucleus (Kalen et al., 1988). The hippocampus provides glutamatergic input into the VTA (Legault and Wise, 2001). In addition, other dopamine-containing neurons within the

midbrain are located in the substantia nigra (SN). These neurons project to the striatum, are vital for coordinated movement, and are involved in the pathophysiology of certain psychiatric and neurological disorders including Parkinson's disease, obsessive-compulsive disorder, and drug addiction (Joel et al., 2005; Robbins and Everitt, 2002). In addition, this projection may be involved in the maintenance of drug-taking after the onset of dependence and contribute to cue-induced relapse (Ito et al., 2002).

Projections from the VTA to the NAcc

Based on the finding that most drugs of abuse are self-administered into the VTA and NAcc, these findings have been most extensively studied (Wise and Hoffman, 1992). A role for these dopaminergic projections in sensitivity to abused drugs is suggested by studies showing that, 1) dopaminergic drugs modulate the effects of abused drugs on locomotion and reinforcement, 2) lesions of dopamine-containing neurons in the VTA block the stimulant and reinforcing effects of abused drugs, and 3) the majority of abused drugs augment dopamine neurotransmission within the nucleus accumbens. Studies supporting the proposed role for dopamine have used various neuroanatomical techniques including lesions, microinjections, microdialysis, electyrophyisology, as well human imaging techniques.

Lesion Studies

Electrolytic and excitotoxic chemical lesions of the VTA and NAcc have been used to investigate the role of these areas in cocaine- and amphetamine-induced locomotor stimulation (Makanjuola and Ashcroft, 1982; Teitelbaum et al., 1979; Woodruff et al., 1976). 6-Hydroxydopamine (6-OHDA), a neurotoxin that specifically destroys catecholamine-containing neurons, provided further support for the role of

dopamine in drug-induced stimulation (Bacopoulos et al., 1979; Breese et al., 1985; Kiianmaa, 1978). Since the neurotoxic action of 6-OHDA requires the presence of the dopamine or norepinephrine transporter, pre-treating animals with a norepinephrine transporter blocker such as desipramine can create dopamine-specific lesions, while pre-treating with a dopamine transporter blocker such as GBR-12909 can create norepinephrine-specific lesions. Using these lesioning techniques, Ventura et al. (2004; 2003) have found that noradrenergic input to the mesolimbic dopamine system is critical for amphetamine reinforcement and stimulation. In the case of ethanol, no studies have measured the effects of these lesions on ethanol stimulation, although some studies have reported an effect of 6-OHDA lesions on the locomotor depressant response in rats (Bacopoulos et al., 1979; Breese et al., 1985). Moreover, studies using 6-OHDA lesions of norepinephrine-containing areas of the midbrain blocked the sedative response to an injection of a high dose (1 g/kg) of ethanol in Wistar rats, but had no effect on the stimulant response to a lower dose (0.1 g/kg) (Mason et al., 1979). Kiianmaa (1978) reported that destruction of the dopamine-containing neurons of the substantia nigra with 6-OHDA was effective in blocking the incoordinating effect of ethanol on the tilting plane task. Although these results suggested that midbrain dopamine is important for ethanol's locomotor effects, 6-OHDA lesions of midbrain dopamine neurons the NAcc have not been used to study ethanol stimulation.

Microinjection studies

A potential mechanism underlying drug reinforcement and stimulation may be net inhibition of neurons within the NAcc. A substantial body of evidence exists suggesting that inhibition of NAcc neurons results in locomotor stimulation, and that drugs and

stimuli that promote this inhibition are reinforcing (for review see Pennartz et al., 1994; Tzschentke and Schmidt, 2000). A common approach is to microinject drugs directly into discrete brain regions through stereotaxically implanted cannulae. This technique is amenable to studying drug sensitivity and drug self-administration. Intra-NAcc injections of dopamine resulted in locomotor stimulation (Pijnenburg and van Rossum, 1973), and data from experiments using dopamine-depleted mice suggested that this stimulation is due to activation of inhibitory D2/D3 receptors in the NAcc (Ross et al., 1988). These findings support the idea that dopaminergic transmission (as well as opioidergic transmission), upon activation by drugs of abuse, would inhibit the NAcc and promote locomotion, as would direct administration of GABA receptor agonists and NMDA receptor antagonists into the NAcc. In support of the idea that inhibition of the NAcc is reinforcing, studies have shown that NMDA receptor antagonists are self-administered by animals into the NAcc (Carlezon and Wise, 1996), although it is unknown whether direct infusion of GABA receptor agonists would support self-administration. Benzodiazepines and barbiturates, which promote GABA-mediated inhibitory transmission (Olsen, 1981; Squires et al., 1984) stimulate locomotor activity (Dudek and Phillips, 1983; File and Pellow, 1985; Phillips et al., 1992), but actually decrease NAcc dopamine transmission (Brose et al., 1988; Finlay et al., 1992; Masuzawa et al., 2003; Zetterstrom and Fillenz, 1990). This raises the possibility that these drugs promote locomotion independently of dopamine, possible by inhibiting NAcc directly to promote drug induced locomotion and reinforcement. However, this idea is contradicted by findings indicating that microinjections of GABA receptor antagonists such as picrotoxin and bicuculline into the NAcc promote locomotion (Wong et al., 1991).

Interestingly, in the aforementioned study, intra-NAcc administration of the $GABA_A$ receptor agonist 3-aminopropane sulphonic acid decreases locomotion at low doses but increased locomotion at high doses. These complicated results may be due to dual actions of GABAergic drugs presynaptically on dopamine terminals in the NAcc, and post-synaptically on NAcc projections neurons. Ethanol may inhibit these neurons directly and indirectly through its ability to stimulate dopaminergic, inhibit excitatory, and potentiate inhibitory neurotransmission (Brodie et al., 1999; Tzschentke and Schmidt, 2000). There are, however, a number of studies that contradict the idea that NAcc inhibition stimulates locomotion (Pennartz et al., 1994). For example, intra-NAcc glutamate agonists (Donzanti and Uretsky, 1983) have been shown to stimulate behavior, as have intra-NAcc injections of GABA antagonists (Wachtel and Anden, 1978).

Microdialysis studies

Microdialysis is a commonly used technique to measure extracellular neurotransmitter levels within the mesolimbic dopamine system. A probe is placed into a particular brain region or into a brain slice. The probe consists of a porous membrane that allows the diffusion of neurotransmitters and other small molecules into a perfusate, usually an artificial cerebrospinal fluid that is collected with the aid of a fraction collector. Using chromatographic techniques, the content of the perfusate are fractionated and quantified by sensitive fluorescence or electrochemical methods. The advantage is that temporal fluctuations in neurotransmitter levels in the interstitial fluid of discrete brain regions can be followed in freely-moving animals. Using microdialysis, researchers found that intra-VTA injections of muscimol that evoked increases in locomotor activity resulted in increases in dopamine levels within the NAcc, thereby

supporting a role for this projection in locomotion (Klitenick et al., 1992; Oakley et al., 1991). In addition, microdialysis studies have found that most drugs of abuse, including psychostimulants, opiates, nicotine and ethanol, increase extracellular dopamine levels in the NAcc (Di Chiara and Imperato, 1988; Yim and Gonzales, 2000). Furthermore, stimuli associated with drug administration can promote increases in extracellular dopamine levels (Duvauchelle et al., 2000). In the case of ethanol, evidence from microdialysis and microinjection studies indicates that ethanol, when administered systemically or directly into the VTA, causes increases in extracellular dopamine levels in the NAcc (Imperato and Di Chiara, 1986; Yim and Gonzales, 2000).

Electrophysiological studies

Electrophysiological analysis of neuronal cell firing in the NAcc has been useful in examining the effects of drugs on changes in neuronal firing and rapid dopamine signaling during drug self-administration. In a study of the reinforcing effects of NMDA receptor antagonists, only antagonists that were self-administered (such as PCP) were effective in activating midbrain neurons (French, 1994). Research from electrophysiological recordings has also provided insights into the microcircuitry of the mesolimbic dopamine system; these recordings have suggested that the NAcc contains neuronal 'ensembles' – groups of neurons with distinct excitatory inputs and specific outputs (Carelli and Wightman, 2004; Pennartz et al., 1994). Further, NAcc neurons often have "up" and "down" states that reflect different levels of excitability (O'Donnell et al., 1999) which can be modulated by drug treatment (Brady et al., 2005). Because a particular firing pattern or neuronal response depends on the neuronal ensemble from

which the recordings are made, these findings may provide an explanation for discrepant findings obtained from different experiments.

Using electrophysiological recordings, Brodie et al. (1999) reported that ethanol directly activates dissociated VTA neurons. Moreover, VTA neurons prepared from DBA/2J mice were more sensitive to direct activation by ethanol than C57BL/6J mice. This may account for the greater sensitivity to ethanol's locomotor stimulant effects in DBA/2J compared to C57BL/6J mice (Brodie and Appel, 2000). Ethanol activation of dopaminergic VTA neurons is consistent with the results of a behavioral study by Rodd-Henricks et al. (2000) showing that rats will self-administer ethanol directly into the VTA. Subsequent work has suggested that ethanol activates the VTA directly through potassium channels (Appel et al., 2003), and possibly through the potentiation of $GABA_A$ receptors (Nowak et al., 1998). Microinjections of low doses of muscimol increased locomotor activity and dopamine in the NAcc (Kalivas et al., 1990). In addition, Boehm et al. (2002a) have shown that intra-VTA injections of baclofen, a $GABA_B$ receptor agonist, modulated the locomotor response to ethanol in FAST mice, suggesting that ethanol may have its effect at the level of the VTA.

Voltammetry Studies

Another way to measure increases in neurotransmitter levels within the synapse is with *in vivo* cyclic voltammetry. This technique relies on the *in situ* oxidation of certain neurotransmitters such as the catecholamines and indolamines (Shellenberger and Gordon, 1971). An oxidizing electrode is implanted into a brain slice or intra-cerebrally via stereotaxic surgery, and neurotransmitter flux is measured as changes in oxidative currents. The sub-second temporal resolution of this technique makes it particularly

useful for self-administration studies (Phillips et al., 2003; Robinson et al., 2003). For example, studies using *in vivo* cyclic voltammetry have shown that increases in dopamine occur in the NAcc during the acquisition of a ICSS task, but not during maintenance of this behavior (Garris et al., 1999). When used in combination with electrophysiogical recordings, the release of neurotransmitters can be directly compared to neuronal firing patterns (Carelli and Wightman, 2004). Studies using *in-vivo* voltammetry have found that ethanol decreases dopamine transporter velocity (Robinson et al., 2005) which may be an additional mechanism of ethanol's ability to promote dopamine release. Studies such as these have provided insights into the role of dopamine signaling during behavioral tasks, thereby leading to more comprehensive theories of dopamine's role in motivated behavior (Everitt et al., 2001; Tobler et al., 2005). However, because only of fraction of extracellular transmitter is oxidized, a major disadvantage of this technique is that measurement of basal neurotransmitter levels is not possible. Therefore, differences in tonic dopamine activity between rodent strains or treatment groups cannot be studied with this technique.

Human imaging studies

In humans, many of the above techniques are too invasive and dangerous to be practical or ethical. However, recent imaging studies have permitted the study of brain functioning during drug administration. Studies using positron emission tomography (PET), which use radioactive ligands to determine receptor and dopamine transporter density, have found that subjective measures of cocaine euphoria were correlated with dopamine transporter occupancy in striatal areas (Volkow et al., 1997). Functional magnetic resonance imaging (fMRI) studies are especially useful in studying the

conditioning processes involved in drug addiction. For example, in smokers, smoking related images induced a greater fMRI signal in mesolimbic dopamine areas, compared to neutral images (Due et al., 2002). Using PET imaging, Boileau et al., (2003) reported ethanol-induced increases in dopamine in the NAcc in humans, confirming findings from *in-vivo* microdialysis in rodents. Also, fMRI has been used in combination with PET to study alcohol craving. Ethanol-associated stimuli activated the PFC and striatum to a greater degree in alcoholics than in controls. In addition, the availability of D2-like receptors in the NAcc was shown by PET to be associated with craving severity and by fMRI to be associated with greater cue-induced activation of the PFC (Heinz et al., 2004).

Dopamine in the core vs. the shell

The sites of action for each of these drugs effects may reside in different substructures of the NAcc. The "shell" of the NAcc extends slightly more ventral and medial than the "core", which surrounds the anterior commissure (Di Chiara, 2002; Gonzales et al., 2004; Paxinos and Watson, 1997). Microdialysis studies have suggested that dopamine innervation of the shell is responsive to the motivational valence and novelty of stimuli, whereas the core is responsive to a larger range of motivational stimuli (Di Chiara, 2002). Drug-induced stimulation and reinforcement are dissociable within the NAcc. 6-OHDA lesions of the NAcc core blocked amphetamine-induced locomotion but not conditioned place preference, whereas the reverse was true for lesions of the NAcc shell (Sellings and Clarke, 2003). However, excitotoxic lesions of the NAcc shell blocked the stimulant response to cocaine, but had no effect on cocaine self-administration (Ito et al., 2004). These data emphasize the importance of attention to

anatomical subregions in the study of drug effects. Systemic injection of ethanol resulted in increases of dopamine in both the shell and core of the mouse NAcc (Zocchi et al., 2003). These results are consistent with a study by Hitzemann and Hitzemann (1997), which found that systemic ethanol injections increase Fos-like immunoreactivity (Fos-li) in both the core and the shell. However, Porrino et al. (1998) found that ethanol consumption increased glucose utilization in the shell but not the core, which may indicate that the method of administration is important for determining the regional effects of ethanol in the NAcc.

Projections from the VTA to the PFC

While the projections from the VTA to the NAcc have received a great deal of attention, the VTA-PFC projections are interesting because they seem to work in opposition to the VTA-NAcc projection. Dopamine transmission within the NAcc and PFC are generally negatively correlated with each other. For example, while 6-OHDA lesions of the NAcc block the stimulant response to amphetamine, similar lesions of the PFC enhance this response (Duvauchelle et al., 2000). This may be due to direct connections from the PFC to the NAcc, or to differential noradrenergic input into these areas (Deckel et al., 1995). Dopaminergic activity within the PFC has been shown to inhibit both the dopaminergic and locomotor response to intra-NAcc injections of amphetamine (Vezina et al., 1991). Psychostimulant-induced increases in glutamate within the NAcc has been established (Reid et al., 1997), and ultrastructural (Sesack and Pickel, 1992) and neurophysiological (Legault and Wise, 2001; Rossetti et al., 1998; Sesack and Pickel, 1992; You et al., 1998) experiments have supported the existence glutamatergic projections from the PFC to the NAcc and VTA. Glutamatergic

connections from the PFC to the NAcc may be inhibited by increases in PFC dopamine, resulting in a decrease in excitatory input into the NAcc and a decrease in NAcc dopamine (Vezina et al., 1991). Darracq et al. (2001) found that infusion of metabotropic glutamate receptor antagonists into the NAcc were effective in attenuating the dopaminergic and locomotor response to amphetamine. These data suggest that the glutamatergic input into the NAcc from the PFC, as well as dopaminergic input to the NAcc from the VTA, is important for drug-induced locomotion.

The role of norepinephrine in the PFC and NAcc is likely to be important for the locomotor response to drugs of abuse as well (Auclair et al., 2004). For example, in rats, the noradrenergic receptor antagonist, prazosin, was effective in decreasing the locomotor response to amphetamine, but not the amphetamine-induced increases in NAcc dopamine (Darracq et al., 2001). Further, α_{1b}-adrenergic receptor knockout mice are insensitive to the locomotor and rewarding effects of cocaine, amphetamine, and morphine (Drouin et al., 2002). Together, these results suggest that a norepinephrine input into the PFC is important in the production of drug-induced locomotor behavior. There are very few studies demonstrating an interaction of ethanol with norepinephrine in the PFC. In fact, systemic injections of ethanol did not increase dopamine within the PFC (Bassareo et al., 1996). Samson and Chapell (2003) used intra-PFC and NAcc injections of dopaminergic drugs in an ethanol drinking paradigm, their results indicate that the PFC is involved with the onset of drinking, whereas the NAcc is involved with its maintenance.

Ethanol's interactions with other systems

The amygdala

Recent studies have also provided evidence for the involvement of the extended amygdala in ethanol-induced locomotion. The major output nuclei of the amygdala, the central nucleus of the amygdala (CeA) has received particular attention. The CeA and other major components of the extended amygdala, including the bed nucleus of the stria terminalis (BNST) and NAcc shell regions, are interconnected with the mesolimbic dopamine system. Anatomical evidence has shown extensive catecholaminergic innervation of the CeA, including dopaminergic efferents from the VTA (Asan, 1998; Fudge and Haber, 2000). Using microdialysis in the rat CeA, Yoshimoto et al. (2000) found increases in both dopamine and serotonin in response to systemic ethanol injections.

Other evidence for the involvement of the CeA was obtained by mapping studies using c-Fos, a protein that is widely expressed in the brain, which can be easily detected in brain sections using appropriate antibodies. Because c-Fos is expressed throughout the brain in response to a wide variety of stimuli, it is thought to be a marker of neuronal activity. Studies using c-Fos mapping have the advantage that, since a drug can induce c-Fos expression throughout the brain, the magnitude of expression can be correlated with behavioral measures. Using this technique, novel ethanol-sensitive brain areas have been discovered (Demarest et al., 1999b; Ryabinin et al., 1997). For example, Hitzemann and Hitzemann (1997) have found differences in Fos-li in the CeA of DBA/2J and C57BL/6J mice, suggesting that differences in ethanol activation of the CeA may underlie the divergent locomotor responses to ethanol in these strains of mice. Interestingly, while ethanol-induced increases in c-Fos expression were observed in the NAcc and striatal areas of these mice, there were no differences between the strains. However, Demarest et

al. (1999b) found greater increases in Fos-li in the CeA of FAST mice, compared to SLOW mice. Furthermore, Fos-li in the VTA of SLOW mice was decreased by ethanol, raising the possibility that ethanol may induce relatively greater sedation in SLOW mice (Shen et al., 1996) through its effects on this brain region. A correlation between ethanol-induced locomotion and Fos-li in the CeA was corroborated by an examination of an F2 intercross of DBA/2J and C57BL/6J mice, in which animals with high locomotor responses to ethanol had larger Fos-li in CeA neurons than animals with low responses (Demarest et al., 1998). However, whether the CeA directly influences ethanol-induced locomotor activity in FAST and SLOW mice has not been investigated.

The opioid system

Activation of opioid receptors on GABAergic interneurons has been found to disinhibit dopamine containing neurons in the VTA (Johnson and North, 1992). Findings that ethanol stimulated increases in β-endorphin in the NAcc (Olive et al., 2000; Rouge-Pont et al., 2002) suggests that the effect of ethanol on the mesolimbic dopamine is mediated by ethanol-induced activation of opioidergic transmission (Gianoulakis, 2001; Herz, 1997). In support of a role for opioids in ethanol-induced locomotion, lesions of beta-endorphin containing neurons in the hypothalamus were found to block the effect of ethanol on locomotor stimulation in Swiss-Webster mice (Sanchis-Segura and Aragon, 2002; Sanchis-Segura et al., 2000), and opioid receptor antagonists were effective as well (Pastor et al., 2005; Sanchis-Segura et al., 2004). However, these findings have not been replicated in FAST and SLOW mice (Holstein et al., 2005; Meyer and Phillips, unpublished data), which may be due to genetic differences in these animals.

Neurosteroid systems

Another set of studies have suggested that ethanol's GABAergic activity may be related to its effects on neurosteroid systems. The $GABA_A$ receptor contains a putative binding site for neuroactive steroids, such as 3α-hydroxy-5α-pregnan-20-one (allopregnanolone), an endogenous metabolite of progesterone (Im et al., 1990; Purdy et al., 1992; Ueno et al., 2004). Acute ethanol administration has been found to increase concentrations of neuroactive steroids that act as positive allosteric modulators of the $GABA_A$ receptor in the brains of certain strains of rats and mice (Barbaccia et al., 1999; Finn et al., 2004; Gabriel et al., 2004; O'Dell et al., 2004). Thus, one proposed mechanism for the effects of ethanol on GABAergic signaling is the induction of allopregnanolone in the brain (VanDoren et al., 2000). Previous studies have found a genetic association between sensitivity to the acute locomotor effect of ethanol and allopregnanolone (Korpi et al., 2001; Palmer et al., 2002a; 2002b; 2002c). The common neural substrate for ethanol and allopregnanolone may be the mesolimbic dopamine system, as allopregnanolone has been shown to increase dopamine levels within the NAcc (Rouge-Pont et al., 2002).

Summary of experiments

While several studies have investigated the neurobiological substrates of ethanol-reinforcement, few studies have examined the neurobiology of ethanol sensitivity in terms of locomotor behavior. The overall goal of this project was to investigate potential brain areas and neurochemical systems responsible for ethanol-induced locomotion. The FAST and SLOW selected mouse lines are particularly useful for this purpose. Given the extensive literature indicating that dopamine plays an important role in ethanol-induced

stimulation, and that ethanol has effects at multiple neurotransmitter receptors that modulate dopaminergic function, it seems likely that the mesolimbic dopamine system is altered in FAST and SLOW mice. The experiments in this project investigated the interaction of ethanol with the mesolimbic dopamine system and the amygdala in these mice, through the use of stereotaxic electrolytic lesioning and brain microdialysis. We hypothesized that these brain areas would be differentially sensitive to ethanol. Also, because dopamine modulates glutamate transmission in the NAcc, and FAST and SLOW mice are differentially sensitive to glutamatergic drugs, we hypothesized that ethanol would differentially regulate glutamate transmission in the NAcc.

While the neural substrates of ethanol-induced locomotor depression (such as that of SLOW mice) have not been extensively studied, these experiments investigated the possible roles of the VTA and NAcc in this behavior as well. It is possible that selective breeding for ethanol-induced locomotor depression resulted in increased sensitivity to ethanol-induced decreases in dopamine levels. Reductions in NAcc dopamine may be a neurochemical mechanism for locomotor depression (Sugita et al., 1989). We tested the hypothesis that VTA and NAcc lesions would attenuate ethanol stimulation in FAST mice and depression in SLOW mice. Further, we tested the prediction that ethanol-induced increases in dopamine and glutamate within the NAcc would be greater in FAST compared to SLOW mice, and would be blocked by VTA lesions.

Methods

Subjects

Originating from a genetically heterogenous stock, FAST and SLOW mice were selectively bred in two replicates for extreme sensitivity to ethanol-induced locomotor stimulation (FAST-1, FAST-2) and depression (SLOW-1, SLOW-2) (Crabbe et al., 1987; Phillips et al., 1991). Only males were used to decrease the number of mice needed to complete these experiments. These mice are bred and maintained in the Portland Veterans Affairs Medical Center animal care colony, and housed in groups of 2-5 in 28 x 18 x 13 (l x w x h) cm clear polycarbonate cages with corn-cob bedding and air-filter lids. Food (Purina Laboratory Rodent Chow; Purina Mills, St. Louis, MO) and tap water were suspended from stainless steel wire lids and were available at all times except during the test sessions. Mice were housed with dam and sire until weaning at 21$\pm$2 days of age, and then housed 2-4 per cage in isosexual groups with mice of the same genotype. Testing occurred between 08:00 h and 16:00 h (the colony lights were on from 06:00 to 18:00). Room temperature was maintained between 20 and 22 °C in the colony and testing rooms. Mice were aged 50 to 100 days and weighed 14 to 30 g at the time of surgery. All procedures were performed in accordance with the Institutional Animal Care and Use Committee and National Institutes of Health guidelines for the care and use of laboratory animals. Experiments were designed in such a way as to minimize suffering and utilize the smallest number of mice as possible. Final group sizes for each experiment are presented in the results section.

Drugs

All drugs were prepared in 0.9% physiological saline (Baxter Healthcare Corporation, Deerfield, IL) except 6-OHDA, which was prepared in 0.1% ascorbic acid dissolved in saline. Ethanol (Pharmco Products, Brookfield, CT) was diluted from 100% to a final concentration of 20 % (v/v). Mice were injected intraperitoneally (i.p.) with 2 g/kg ethanol by varying the volume of injection, depending on the weight of the mouse. This dose was chosen because FAST and SLOW mice were selectively bred based on their responses to this dose of ethanol (Crabbe et al., 1987; Phillips et al., 1991). Cocaine HCl (40mg/kg; Sigma, St. Louis, MO) was injected i.p. at volumes of 10 ml/kg. The 40 mg/kg dose was chosen because previous studies in our laboratory have shown that FAST and SLOW mice are differentially sensitive to this dose (Bergstrom et al., 2003). Stock ketamine/xylazine/acepromazine was purchased from the Portland Veterans Affairs Medical Center pharmacy, and contained 5 ml ketamine (100 mg/ml), 2.5 ml xylazine (20 mg/ml), 1.5 ml sterile NaCl solution, and 1 ml acepromazine (10 mg/ml). This stock solution was diluted 1:6 in saline for injection. Desipramine and pargyline were purchased from Sigma, dissolved in saline, and injected at a dose of 25 mg/kg in volumes of 10 ml/kg. 6-OHDA was dissolved in 0.1% ascorbic acid (Sigma), and injected as described in the surgical procedures section.

Activity monitors

Mice were tested in clear acrylic plastic boxes (40 cm long x 40 cm wide x 30 cm high), covered by plastic lids with 0.64-cm diameter holes for ventilation. These boxes were placed in automated activity monitors (Accuscan Instruments, Columbus, OH), which consisted of 8 pairs of intersecting infrared photobeams, located 2 cm above the

cage floor. Occlusions of these photobeams were used to calculate the distance traveled (in cm) by a mouse during the test sessions. The activity monitors were housed in individual, opaque sound attenuation chambers (Flair Plastics, Portland, OR) that also contained a 15 W fluorescent bulb, and a fan that provided ventilation and masked background noise.

Experiment 1

Surgery

Each mouse was anesthetized with ketamine/xylazine/acepromazine cocktail according to the following equation:

$$\text{Injection vol (ml)} = 2 \times (((\text{body weight (g)})/100)-0.08)$$

Final doses were approximately 141.7 mg/kg ketamine, 14.2 mg/kg xylazine, and 2.8 mg/kg acepromazine. This injection anesthetized (i.e., non-responsive to a moderate paw-pinch) FAST and SLOW mice for approximately 90 min. After injection, mice were left undisturbed for 10 min, and earpunched for identification. Then, a small circular area of the scalp (~15 mm diameter) was removed with surgical scissors, and the wound was disinfected with a cotton swab soaked in 100% ethanol. The front teeth were inserted into the bite bar of the stereotaxic surgery stage (Cartesian Research, Sandy, OR), and a small nose cone secured the mouse's snout. A cotton swab was used to move the tongue away from the bite bar so that the mouse would not suffocate. The mouse's head was further stabilized by placing an ear bar into each ear canal.

Once inserted into the stereotaxic stage, a magnifying scope was used to locate the major landmarks of the brain: bregma, lambda, and the saggital suture. The Cartesian origin was defined as the intersection between bregma and the saggital suture. A digital coordinate system (Anilam, Jamestown, NY) was used to measure the distance (in mm) from bregma to lambda. Paxinos and Watson (1997) constructed their mouse brain atlas based on a C57BL/6J mouse; the average bregma-lambda distance in this mouse was found to be 4.21 mm. By dividing the measured bregma-lambda distance from the average obtained by Paxinos and Watson, an "adjustment factor" was obtained. This measure was used as an estimate of the mouse's brain size, which was used to calculate adjusted coordinates. Using this atlas, the coordinates of the target brain areas were obtained (in mm, relative to bregma): VTA: 3.5 caudal, 0.6 lateral, 4.5 ventral; CeA: 2.5 caudal, 2.5 lateral, 4.5 ventral; NAcc: 1.4 rostral, 1.0 lateral, 4.5 ventral. For each mouse, these coordinates were multiplied by the "adjustment factor". In this manner, adjusted coordinates for each mouse were obtained, which theoretically compensated for variations in brain size from mouse to mouse.

After the adjusted coordinates were obtained, each mouse's head was leveled using a miniature level (Cartesian Research). Using a 27 gauge drill bit (Cartesian Research), a hole was drilled in the skull above each side of the targeted brain area (VTA, CeA, or NAcc; only one brain region was lesioned per mouse). Miniature steel electrodes with 0.25 mm exposed copper wire (Rhodes Medical Instruments, Summerland, CA) were lowered to the depth of the target brain area. A lesion making device (Ugo Basile, Italy), was used to create electrolytic lesions of the target brain area. Electrical leads were attached to the electrode for lesions and the mouse's ear for

grounding. For the VTA, 0.25 mA was applied for 5 seconds; for the CeA, 0.5 mA was applied for 10 seconds; and for the NAcc, 0.5 mA was applied for 15 seconds. These parameters, chosen from pilot studies, created partial lesions of the VTA and NAcc, extending from 0.15 to 1 mm on the rostro-caudal axis. Larger lesions were avoided because they tended to result in aphagia and severe hypoactivity. Lesions of the CeA typically damaged surrounding areas as well. All lesions were performed bilaterally, and the left-right order of the lesions was counterbalanced between animals. Electrodes were cleaned with a cotton swab soaked in 100% ethanol upon removal from the brain. Two sham groups were included in these studies. Sham-penetrated mice underwent the same procedure except that no current was applied to the electrode. This group was included to determine the effect of any damage caused by the electrode penetrating the brain. For sham-intact mice, the electrodes were not inserted at all, although the holes above the target brain areas were drilled. This group was included as a neurologically intact control group for comparison to the other groups. After surgery was completed, the syringe was removed and the incision was sealed with Durelon dental acrylic (3M, St. Paul, MN). Mice were allowed to recover 7-21 days before behavioral testing began.

The NAcc was lesioned with 6-OHDA in a subset of FAST and SLOW mice. To achieve this, mice were pretreated 30 min prior to surgery with 25 mg/kg desipramine, to prevent transport of 6-OHDA into norepinephrine containing neurons, and 25 mg/kg pargyline, to enhance the effectiveness of 6-OHDA. Surgery was conducted as described above, except that instead of inserting the electrodes, a 1 µl Hamilton syringe (Reno, NV) containing either 8 mg/ml 6-OHDA or 0.1% ascorbic acid vehicle was lowered to the target brain area using a stereotaxic syringe holder (Cartesian Research). 0.5 µl was

injected over the course of 2 min into each side of the target brain area. After each injection, the syringe was left in place for an additional 2 min to allow diffusion of the 6-OHDA. Due to the small number of animals, ethanol-induced behavior was not tested in these mice. The purpose of this experiment was to verify that the dopamine transporter immunostaining procedure described below was able to detect decreases in dopamine terminals caused by injections of 6-OHDA.

Activity testing procedure

After recovery, mice underwent a four-day testing period. On each day, mice were moved, in their home cages, from the colony room to the testing room 45-60 min before testing began, in order to maximize their habituation to the testing environment. On days 1-3, mice were weighed, injected with saline, and placed into the activity monitors for 20 min. Data were collected in 5-min epochs. After the testing session, mice were removed from the activity monitor and placed in their home cages. On day 4, mice were weighed, injected with 2 g/kg ethanol, and placed into the activity monitors for 20 min.

Blood ethanol concentration

On the last testing day, upon removal from the activity monitors, 20 µl of blood was obtained from the retro-orbital sinus using glass capillary tubes (Fisher, Pittsburgh, PA). Each blood sample was placed in a microcentrifuge tube containing 50 µl of 5% zinc sulfate (Sigma), 50 µl of 0.3N barium hydroxide (Sigma), and 300 µl of distilled deionized water. Samples were then centrifuged, and the supernatant was tested for ethanol content using a gas chromatograph (Model HP 5890, Agilent Technologies) with flame ionization detection. Blood ethanol concentrations were extrapolated from an

external standard curve constructed using known concentrations of ethanol (Gallaher et al., 1996).

Histology

After completion of the behavioral experiments, mice were sacrificed by cervical dislocation, decapitated, and their brains were removed using scissors and a pair of sharp forceps. Most brains were placed in cold isopentane for 20 seconds, chilled by a slurry mixture of dry-ice and isopropyl alcohol (Sigma). Brains for immunohistochemistry were placed in 5 ml of 4% paraformaldehyde in phosphate-buffered saline (Sigma) for 48 hours, and then transferred to 5 ml of 20% sucrose in phosphate-buffered saline (Sigma) for at least 48 hours.

The thionin staining procedure was adapted from previous experiments in our laboratory (Boehm et al., 2002a). Frozen brains were mounted in a cryostat (Leica CM1850, Bannockburn, IL) with tissue embedding media (Sakura Finetek, Torrance, CA) and cut in 50 μm coronal sections. In some VTA-lesioned animals, saggital sections were cut, as we have found that this facilitates the determination of the extent of the lesion location on the rostrocaudal axis. Sections through each lesioned brain area were mounted on frosted microscope slides (VWR, West Chester, PA) and allowed to dry for two hours. For thionin staining, slides were submerged in 500 ml of the following solutions: citrisolv (2 min; Fisher), 100% ethanol (2 min), 95% ethanol (2 min), 70% ethanol (2 min), 50% ethanol (2 min), deionized H_2O (3 min), 0.1 mg/ml thionin (50 seconds; Sigma), 70% ethanol (2 min), 95% ethanol (2 min), 100% ethanol, and citrisolv (2 x 1 min). Slides were then coverslipped using cytoseal-60 (Apogent, Kalamazoo, MI) and cover glass (Fisher). Brain sections were inspected at 2.5x magnification using a

Leica microscope (Model CM1850) attached to a SPOT Insight digital camera and software (Diagnostic Instruments, Sterling Heights, MI), and lesion locations were determined by areas of reactive gliosis caused by the lesion (see figure 3). The precise location of the lesions was recorded in Cartesian coordinates according to Paxinos and Watson (1997).

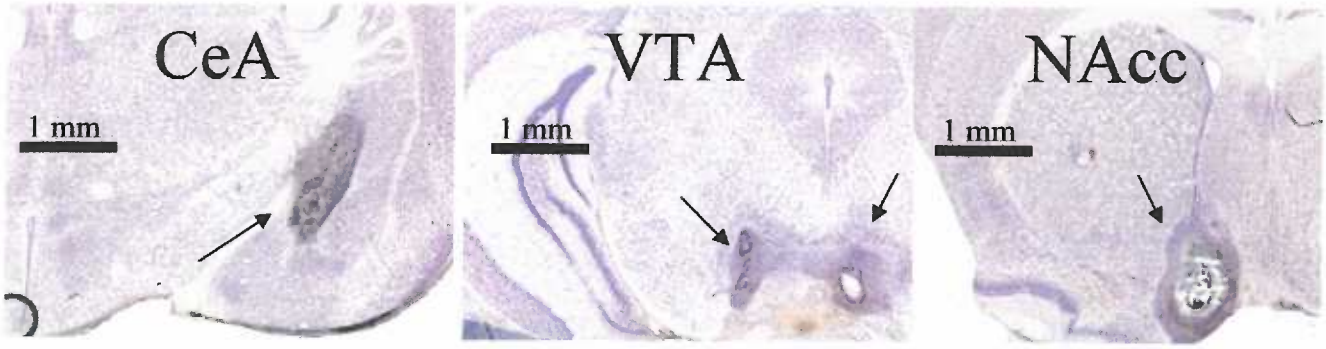


Figure 3: Coronal brain sections showing the extent of damage caused by electrolytic lesions. Extent of the lesions are indicated by the extent of reactive gliosis (arrows, dark purple staining).

Immunohistochemistry

For immunohistochemistry, fixed brains were cut on a cryostat as described above, but alternating sections from the NAcc were placed in 10 mM phosphate buffered saline (PBS; Sigma). Using protocols adapted from Hitzemann et al. (2003), the sections were stained for either dopamine transporter (DAT) or tyrosine hydroxylase (TH), which have been shown to be somewhat specific markers for dopamine-containing neurons (noradrenergic neurons also contain TH). Endogenous peroxidase in red blood cells was inactivated by rinsing the sections in the following solutions, each for three 10 min periods: 1.5% hydrogen peroxide (Sigma; diluted from 30% in 10 mM PBS), 10 mM PBS, 1.5% hydrogen peroxide, 10 mM PBS. Sections were transferred to 1.5 ml microcentrifuge tubes (Fisher), agitated for 1.5 to 2 hours at room temperature in the

following blocking solution: 30 µl rabbit serum (Vector Laboratories, Burlingame, CA), 25 µl 10% Triton X-100 (Sigma), 935 µl 10 mM PBS. 1.0 µl of primary DAT antibody (Oncogene Science Inc, Cambridge, MA) or TH (Chemicon International, Temecula, CA) was then added to each tube, and the tubes were agitated for 48 hours at 4 °C.

For secondary antibody staining, sections were rinsed in 10 mM PBS, and agitated for 1.5 to 2 hours in microcentrifuge tubes containing 5 µl of secondary antibody (anti-goat IgG purified from a rabbit host), 30 µl of rabbit serum, 30 µl of 10% triton X-100, and 935 µl of 10 mM PBS. After agitation, sections were rinsed in 10 mM PBS, and agitated for 1.5 to 2 hours in tubes containing a horseradish peroxidase avidin-biotin complex solution (Vectastain ABC, Vector Laboratories), consisting of 9 µl avidin, 9 µl biotinylated horseradish peroxidase, 30 µl of 10% Triton X-100, and 952 µl 10 mM PBS. This solution was pre-incubated for 30 min before the tissue was added, in order to stabilize the avidin-biotin complex.

After this agitation period, sections were rinsed in 10 mM PBS, incubated in a diaminobenzidine solution (50 mg in 100 ml of 0.1 M Tris, and 1 ml nickel ammonium sulfate, pH = 7.4) for 10 min. The chromatic reaction was initiated by the addition of 35 µl of 30% hydrogen peroxide. The reaction was stopped after 15 seconds by rinsing in 0.1M Tris. The sections were mounted on slides and dehydrated in a graded ethanol series: 70% ethanol (2 min), 95% ethanol (2 min), 100% ethanol, and citrisolv (2 x 1 min). Slides were then coverslipped with cytoseal-60.

DAT immunoreactivity in the NAcc core and shell was quantified in 4 sham-operated and 4 VTA-lesioned mice, using an adaptation of an optical density method described previously (Touchon et al., 2004). For each mouse, three to four photographs

of different sections of the NAcc were taken using a Zeiss Axioplan light microscope (Carl Zeiss, West Germany), as near to 1.34 mm anterior to bregma as possible, as judged by a mouse brain atlas (Franklin and Paxinos, 1997). The optical density of the NAcc core (just ventral to the anterior commisure), shell (medial to the anterior commissure), and the anterior commisure was measured using Image Pro-Plus software (version 3.0, Media Cybernetics, Silver Springs, MD). The optical density of the anterior commisure was subtracted from that of the core and shell, to correct for variations in background staining, and the values obtained for the 3-4 sections were averaged for each mouse. Photographs from each group were taken at the same time, with the same lamp setting, and analyzed for optical density simultaneously.

Experiment 2

Surgery

The surgical, microdialysis, and high pressure liquid chromatography (HPLC) procedures described below were adapted from previous studies in this and other laboratories (Boehm et al., 2002a; McKee and Meshul, 2005; Meshul et al., 1999; Olive et al., 2000). Surgery was begun as described in experiment 1, but instead of the lesioning procedure described, holes were drilled above the left NAcc for insertion of the guide cannula (shaft length 7 mm, outer diameter approximately 0.4 mm) and approximately 2.5 mm caudal and 2.0 mm right of bregma for the fastening of a 20 gauge anchor screw (Small Parts, Inc., Miami Lakes, FL). The anchor screw hole was widened with a 20 gauge hand drill (Cartesian Research), and the anchor screw was inserted until just secure. Using a stereotaxic insertion tool, a plastic CMA/7 guide cannula (CMA Microdialysis, Stockholm, Sweden) was surgically placed above, but not into, the NAcc,

using the following coordinates (relative to bregma): 1.4 mm rostral, 1.0 mm lateral, and 2.9 mm ventral. A stainless steel stylette was inserted into the cannula to prevent clogging. Cannula were secured into place with dental acrylic, and allowed to dry before removal from the stereotaxic stage. At this point, a tethering post (Instech Laboratories, Plymouth Meeting, PA) was cemented to the skull with dental acrylic. A diagram of a completed cannula implantation is shown in figure 4. Mice were allowed to recover for 3-14 days before microdialysis began.

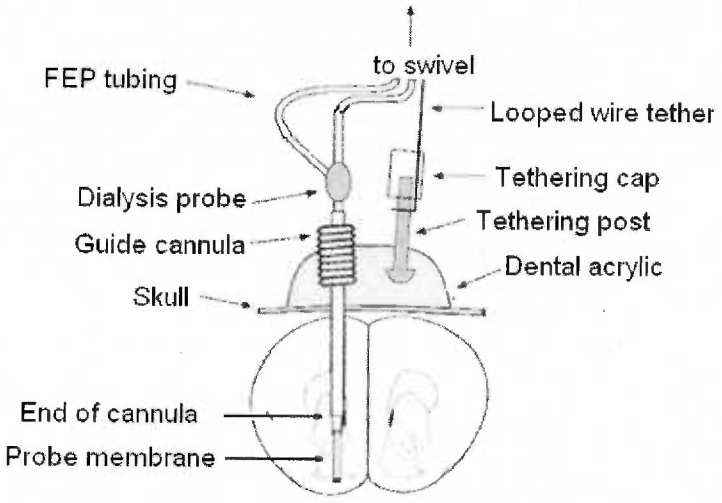


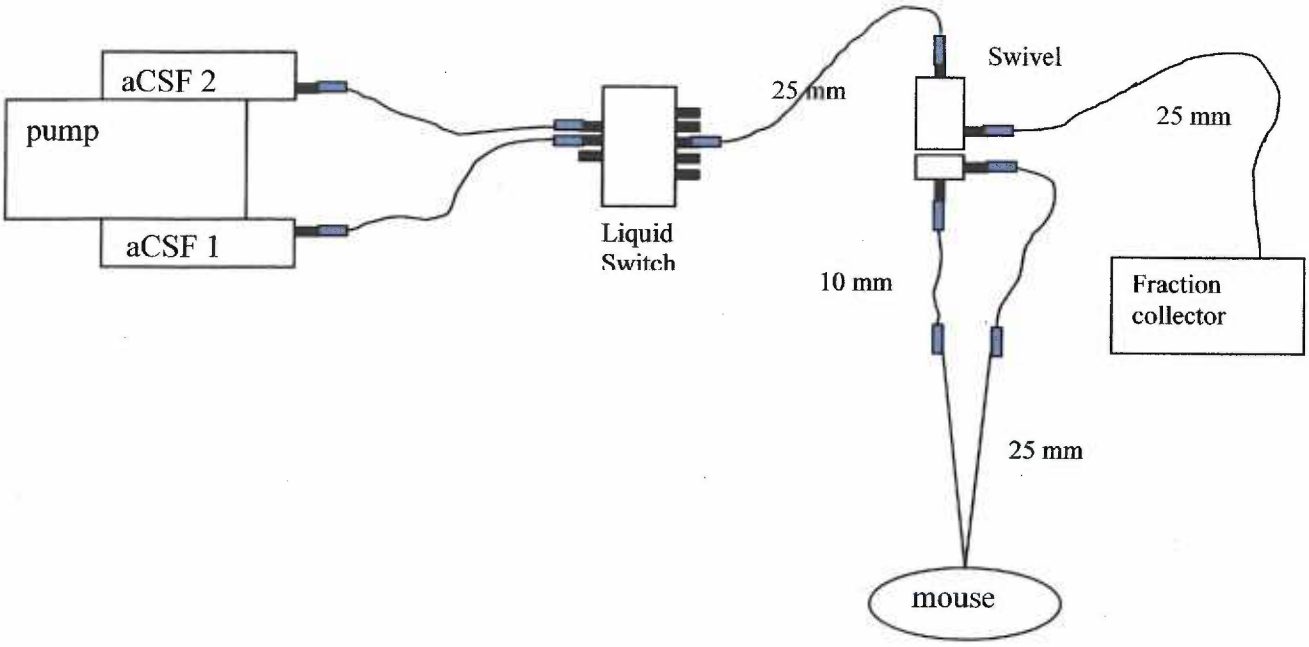
Figure 4: Schematic of a cannulated mouse brain with probe inserted and tether attached. Diagram was adapted from Olive et al., (2000).

Microdialysis set-up

On the evening before testing began (between 3pm and 8pm), one FAST and one SLOW mouse were moved to the testing room, weighed, and lightly anesthetized with ketamine/xylazine/acepromazine mixture according to the following formula:

Injection vol (ml) = 0.0075 ml/g x body weight (g)

This is a sub-hypnotic dose of anesthesia which sedates the mouse long enough to insert the probe and attach it to the wire tether. After sedation, the stylettes were removed, and CMA/7 concentric microdialysis probes (CMA; 6 kDa cut-off; 0.24 mm outer diameter; 1 mm exposed cuprophane membrane) were then inserted into the cannulae. Mice were then tethered to a dual-channel microdialysis swivel (Instech) via a wire attached to the tethering post, and the swivel was attached to a counterbalanced lever arm (Instech) mounted on the activity chamber so that the swivel was suspended above the middle of the chamber (see figure 5). The inlet and outlet channel of the swivel were connected to the probe tubing using polyethylene (PE) tubing. Artificial cerebral spinal fluid (aCSF) containing 145 mM NaCl, 2.8 mM KCl, 1.2 mM CaCl, 1.2 mM $MgCl_2$, and 5.4 mM D-glucose (all from Sigma), was delivered to the dialysis probe using a 2.5 ml glass syringe (CMA) at a rate of 2 µl/min, using a microdialysis pump (CMA) coupled to the swivel via PE tubing. Because we have found that aCSF with pHs higher than 5.6 promote the spontaneous oxidation of dopamine (unpublished data), the pH was adjusted to 5.6, which has been done in previous studies (Yim and Gonzales, 2000). This prevented the oxidation of dopamine in the microdialysis tubing. A liquid switch (CMA) between the pump and the swivel was used to switch between two types of aCSF (see below). The outlet channel of the swivel was connected to a fraction collector (CMA) with PE tubing, and 30-40 µl dialysate samples were collected. The lengths of the tubing connecting the various components are depicted in figure 5. With this set up, fluid takes 6.5 min for liquid to travel from the liquid swivel to the probe, and another 6.5 min to travel from the probe to the fraction collector.



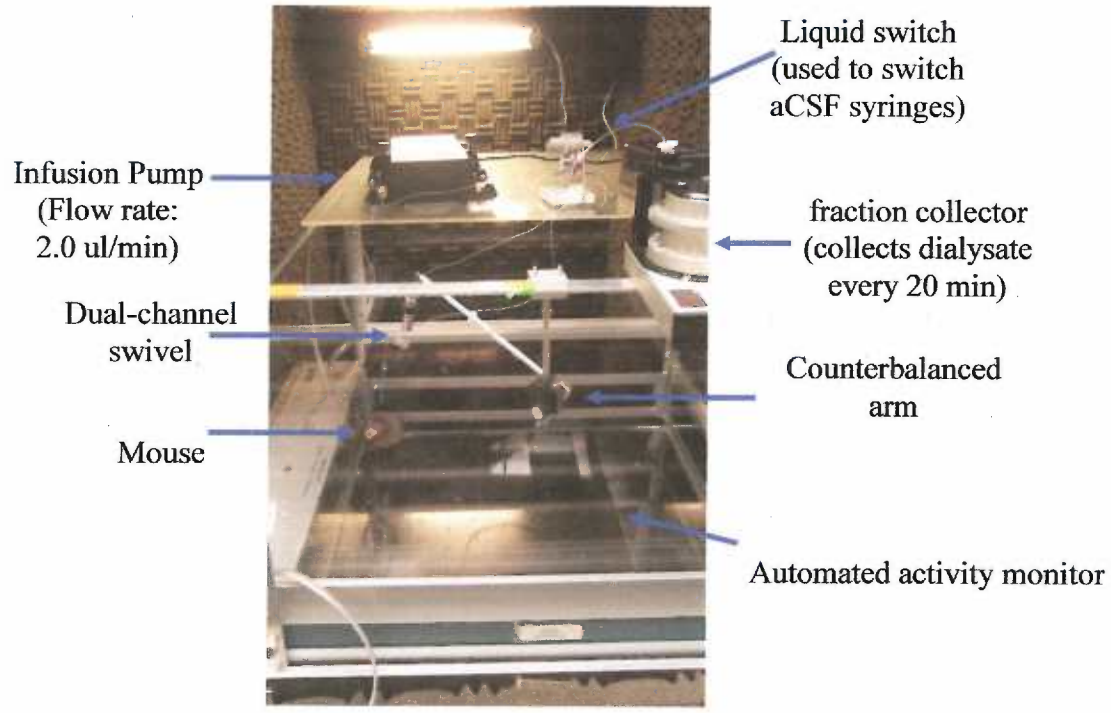


Figure 5: Schematic of microdialysis set-up. Photo shows a mouse attached to the liquid swivel and counterbalanced lever arm.

Once attached to the microdialysis set up, mice were placed into 40 x 40 x 30 cm (l x w x h) clear polycarbonate cages with corn-cob bedding and air-filter lids, which were in turn placed into the activity monitors. A 20 x 20 cm hole was cut in the filter top in order to allow the mouse and swivel to move freely about the cage while preventing escape from the top. Sources of rodent chow and tap water were provided overnight.

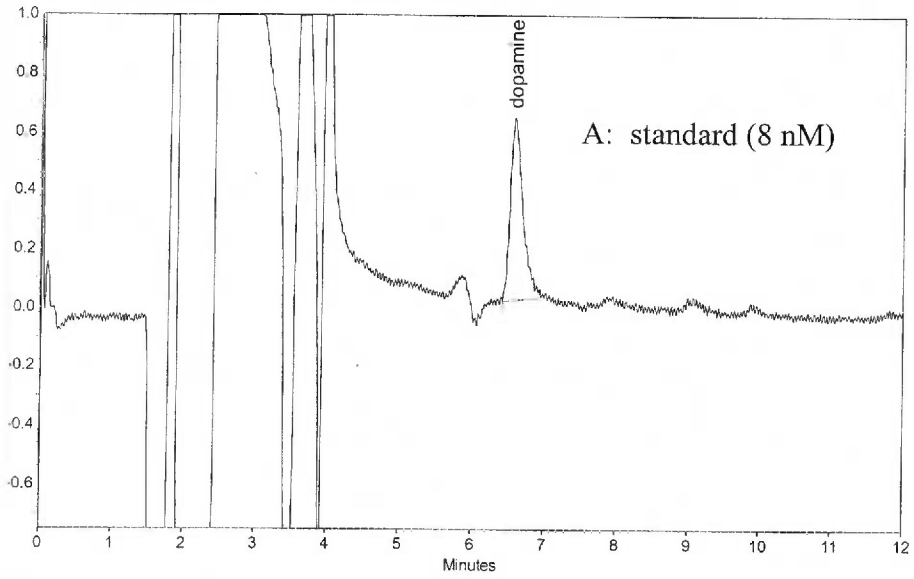
Microdialysis procedure

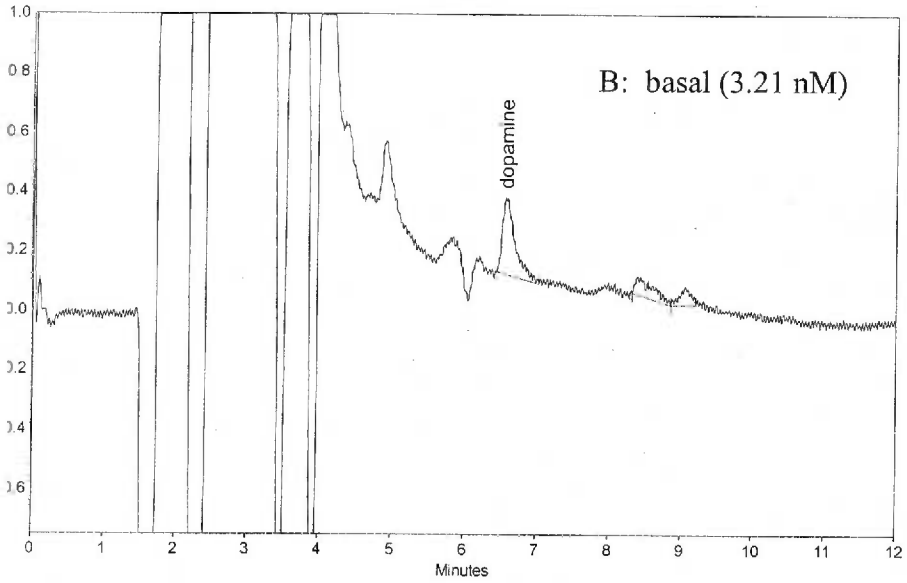
Experimental testing and sample collection began 12-16 hours after probe implantation. To initiate testing, the mice were placed directly into the activity monitors, and the cages, rodent chow, and water were removed. Dialysate sample collection began 6.5 min after activity testing began, to account for the time required for dialysate to travel from the probe to the fraction collector. Dialysate was collected in 20-min epochs in 0.4 ml glass microvials (Agilent Technologies, Palo Alto, CA), sealed with mini crimp-tops (Agilent Technologies). Each vial contained 2 µl of a solution containing 20 mM oxalic acid and 2 M acetic acid (Sigma), to prevent the spontaneous oxidation of dopamine. Since the sensitivity of the HPLC assay dictates the amount of sample needed, we chose 20-min fractions to provide a sufficient amount of sample while still offering some temporal resolution. After one hour of basal activity testing, each mouse was removed from the activity monitors, injected with saline, and returned to the activity monitors. After an hour of post-saline sample collection, each mouse was injected with 40 mg/kg cocaine and returned to the monitors. Finally, 53.5 min after the cocaine injection, the liquid switch was used to switch from normal aCSF to a high potassium containing aCSF. High potassium aCSF was osmotically similar to normal aCSF, except the KCl was increased to 100 mM and the NaCl was decreased to 51.8 mM. The high potassium

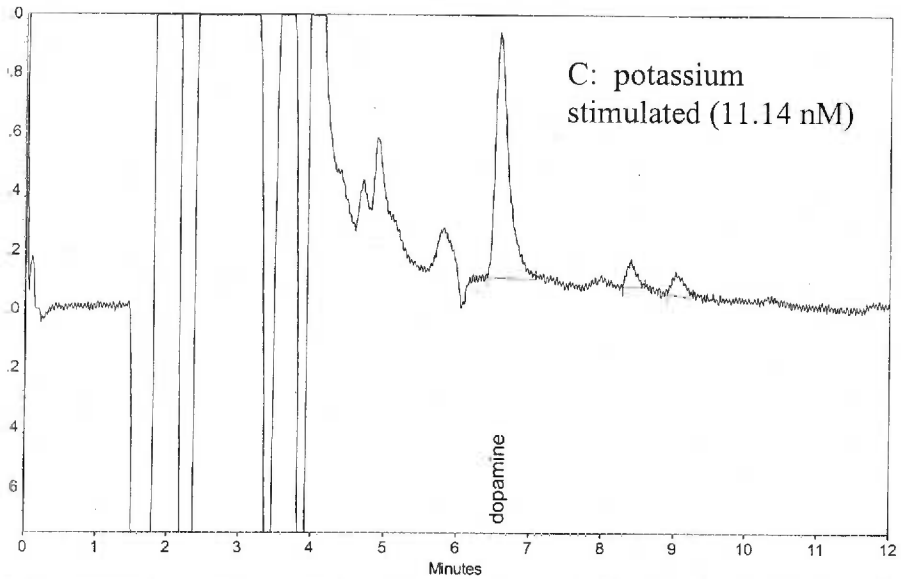
aCSF was perfused for 20 min, and then switched back to normal aCSF for the remaining 51.5 min of the experiment. Dialysate samples were frozen at -40°C until analyzed by HPLC.

High Performance Liquid Chromatography (HPLC)

Dopamine levels in the dialysate fractions were measured using HPLC coupled with electrochemical detection, as described previously (McKee and Meshul, 2005; Olive et al., 2000). An ESA 582 isocratic solvent delivery system (ESA Inc, North Chelmsford, MA) was used to pump mobile phase (10% Acetonitrile, 90 mM sodium phosphate, 50 mM citric acid, 1.7 mM octanesulfonic acid, 50 μM ethylenediaminetetraacetic acid, pH: 5.6) at a flow rate of 0.34 - 0.6 ml/min. This flow rate was varied from subject to subject in order to promote separation from other oxidizable substances, and to compensate for changes in elution times which occurred as a result of gradual column degradation. With these conditions, dopamine metabolites were not measurable. For electrochemical detection using the ESA electrochemical detector (Model Coulochem III), the reducing electrode was set at –100 mV and the oxidizing electrode was set at either 200 or 280 mV. 20 μl samples were injected onto a C18 column (ESA model MD-150, 3-mm inner diameter, 150 mm long, 3-μm particle size) using an ESA 542 autosampler. The column temperature was between 27-35 °C. Column temperature was varied between subjects in order to promote separation of dopamine from other substances. Dialysate levels of dopamine were calculated from a dopamine standard curve (0.15 to 14 nM), prepared at the time of sample collection. The detection limit of this assay was greater than 50 fmol. Examples of HPLC traces obtained using this method are shown in figure 6.







e 6: Chromatograms obtained from HPLC analysis of a standard solution, a basal dialysate sample, and dialysate sample ed after potassium stimulation (top, middle, and bottom panels, respectively). Numbers in parentheses denote the concentration amine represented the dopamine peak in each chromatogram.

Glutamate concentration in dialysate fractions was determined using a Hewlett Packard 1090 interfaced with a Hewlett Packard 1046A fluorescence detector (Agilent Technologies), as described previously (McKee and Meshul, 2005). Samples were derivatized 1 min before injection with o-phthalaldehyde (Sigma) by adding 1 μl of sample, 5 μl of borate buffer (pH 10.4) and 1 μl of o-phthalaldehyde. The entire reaction mixture was injected onto a reverse-phase C18 column (Agilent Technologies) and o-phthalaldehyde derivatives separated using a 5-min linear gradient (flow rate: 0.45 ml/min) of two mobile phases. Mobile phase A contained 0.018% (v/v) tetraethylammonium, 0.3% (v/v) tetrahydrofuran and 20 mM sodium acetate buffer, pH 7.2. Mobile phase B contained 40% (v/v) acetonitrile, 40% (v/v) methanol and 20% (v/v) 100 mM sodium acetate, pH 7.4 (all mobile phase purchased from Sigma). The o-phthalaldehyde derivatives of glutamate were detected by fluorescence using an excitation wavelength of 340 nm and an emission wavelength of 450 nm. Standard solutions, prepared at the time of sample collection, contained 0.125 to 5 picomoles/μl. The detection limit of this assay was greater than 50 fmol. Examples of HPLC traces obtained using this method are shown in figure 7.

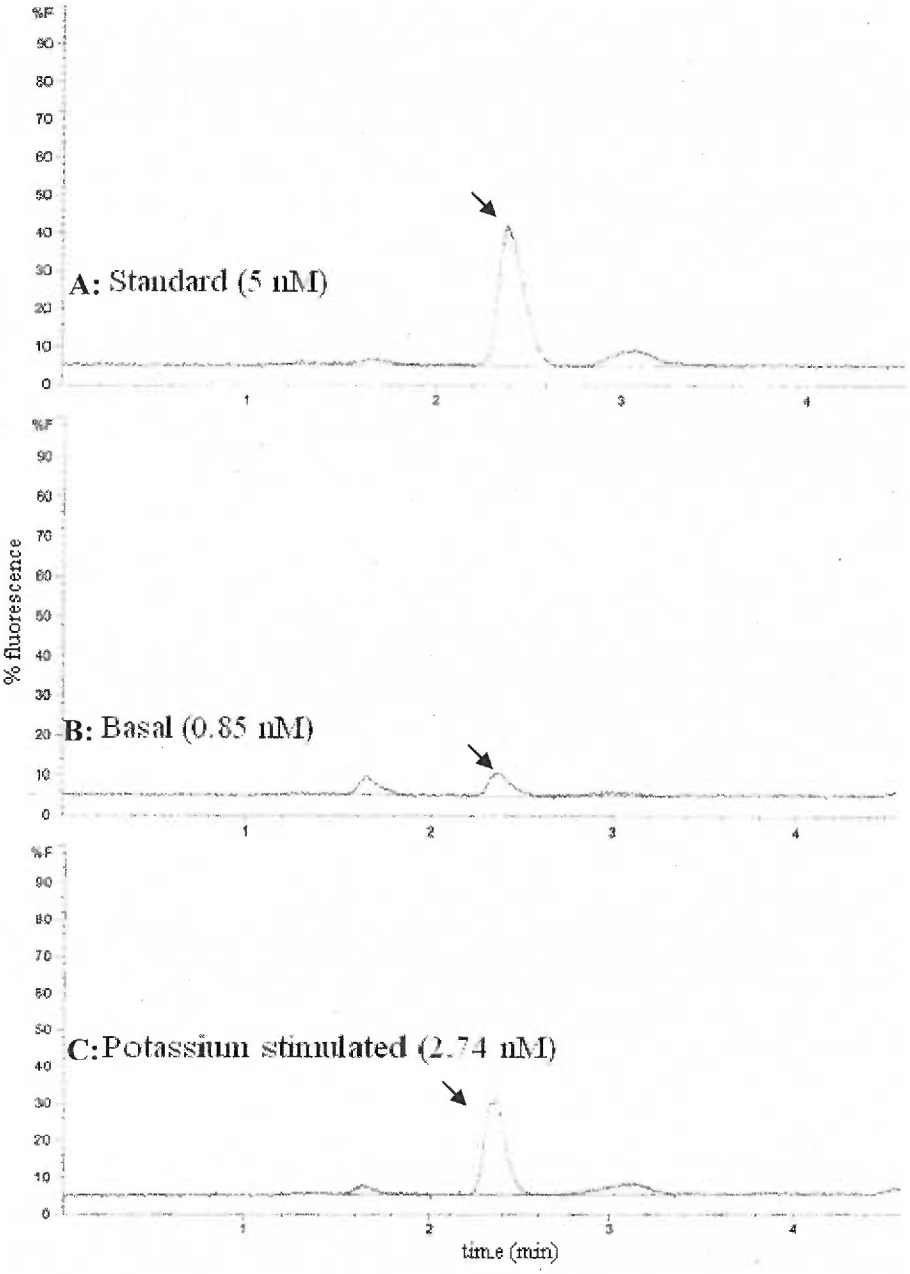


Figure 7: Examples of HPLC traces obtained during analysis of glutamate. Top, middle, and bottom traces are from a standard solution, basal dialysate sample, and a dialysate sample collected after potassium-stimulation, respectively. Glutamate peaks, indicated by arrows, had a retention time of approximately 2.2 min in this assay. Numbers in parentheses refer to the concentration of glutamate represented by the peak.

Histology

After removal of the animal from the activity chambers, and immediately before brain dissection, the probe tubing was cut, and a 1 ml plastic syringe containing methylene blue (Sigma; 10 mg/ml in saline) was attached to the probe inlet tubing, and approximately 20-40 µl of methylene blue was injected. This clearly marks the placement of the probe and allows for very accurate histological analysis. Brains were processed as described in experiment 1, and pictures of the brains sections (50 µm thick cryostat sections) were taken before (figure 8, left panel) and after (right panel) staining with thionin. If a probe was not at least 50% within the boundaries of the NAcc, the data from that mouse were excluded for the entire experiment.

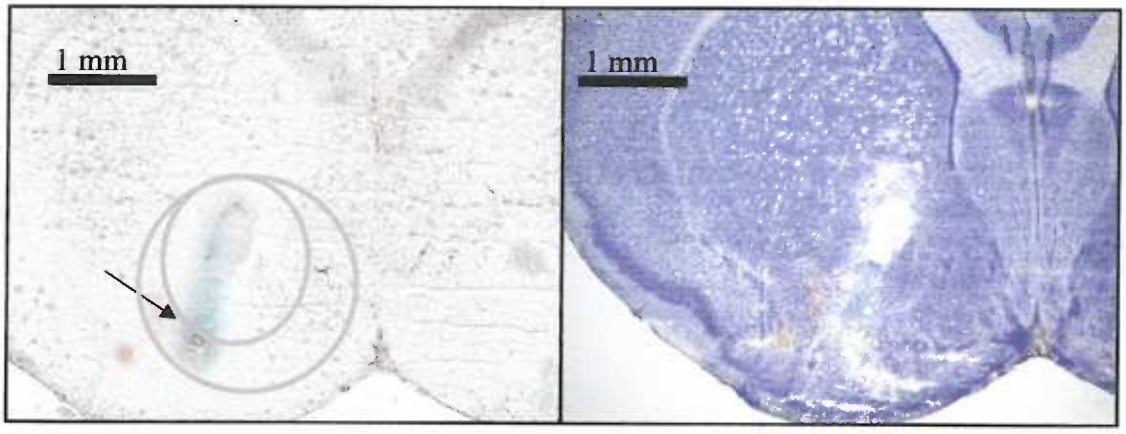


Figure 8: Brain sections, before and after staining with thionin, showing the location of the microdialysis probe as marked by methylene blue (arrow). Inner and outer grey circles denote the approximate boundaries of the NAcc core and shell, respectively.

Experiment 3

The protocol for this experiment was similar to that for experiment 2, except that mice were injected with 2 g/kg ethanol, and samples were collected in 15-min epochs, due to the rapid time course of ethanol's effects on behavior and dopamine levels. Also, the 100 mM potassium perfusion was shortened to 15 minutes so that the length of the perfusion would equal the length of one dialysate collection period.

Experiment 4

Because previous data from our laboratory suggested that the response to ethanol is slightly larger in FAST-2 mice, compared to FAST-1 mice (Boehm et al., 2002a; Palmer et al., 2002a; Phillips et al., 2002), we determined the effects of electrolytic lesions on ethanol-induced increases in NAcc dopamine in the FAST-2 line. Surgery was conducted as described in experiments 1 and 2, except that both lesion and cannulation procedures were conducted simultaneously. Lesioned, sham-penetrated, and sham-intact groups were included in this study. Mice were allowed to recover 7-10 days before being tested in the microdialysis procedure described in experiment 3. Histological analysis of VTA lesions was conduced as described in experiment 1.

Statistics

For body weight data in experiment 1, the effect of the lesions on body weight during the course of the experiment was analyzed with repeated-measures analysis of variance (ANOVA), with Line (FAST, SLOW), Replicate (1, 2), and Lesion (Sham,

Lesion) as between groups factors and Day as the repeated measure. The effects of the lesions on locomotor activity on day 1 and blood ethanol concentrations (BECs) were also analyzed using ANOVA. For ethanol-induced locomotion, data were expressed as "ACT scores", which were equivalent to the distance travelled in response to ethanol on Day 4 minus the response to saline on Day 3. In this manner, positive numbers reflect ethanol-induced stimulation, and negative numbers reflect locomotor depression.

Locomotor activity during the microdialysis studies was compared using ANOVA, with Line (FAST, SLOW) and Replicate (1, 2) as between-groups factors. For the cocaine experiment, the dependent measure was distance travelled during the first 60 min after cocaine injection. For the ethanol experiment, only the first 15 min was analyzed. These time periods were chosen because previous studies have suggested that the peak locomotor responses to cocaine and ethanol occur within 60 and 15 min, respectively (Delfs et al., 1990; Phillips et al., 1991; Porrino, 1993).

For dialysate data, dopamine and glutamate concentrations were expressed as percent relative to the average of the four post-saline samples, and analyzed with ANOVA. The dependent measures were the average change in dialysate levels over the first 60 min after drug administration. For experiment 2, this corresponded to the average change during the three 20-min time periods after cocaine injection. For experiment 3, this was the average change during the four 15-min time periods after ethanol injection.

For experiment 4, data were expressed as percent relative to saline, as in experiment 3. Student's t-tests were used to examine the effect of Lesion (Sham, Lesion) on the first 15 min of ethanol-induced activity and the average change in dialysate dopamine and glutamate levels during the four 15-min post ethanol time periods.

Results

Experiment 1

Activity

There were no differences between Sham-penetrated and Sham-intact controls for any brain area, so these groups were combined for all analyses. Also, there were no interactions between Replicate (1, 2) and Lesion (Sham, Lesion) for any brain area. Therefore, data are presented collapsed across replicate in figure 9 for clarity. Lesions differentially affected ACT scores in FAST and SLOW mice. This was supported by ANOVA, which revealed a significant Line (FAST, SLOW) by Lesion (Sham, Lesion) interaction [$F(1, 79) = 4.07$, $p < 0.05$] that did not interact with replicate. For this reason, and because ethanol has opposite effects on locomotion in FAST and SLOW mice, the effects of lesions in FAST and SLOW mice were evaluated separately.

Of the three brain areas, only VTA-lesions in FAST mice significantly altered the ACT score (figure 10). The effect of the lesion was supported statistically by a main effect of Lesion (Sham, Lesion) for the ACT score [$F(1, 46) = 5.54$, $p < 0.05$]. Activity of the sham and VTA-lesioned mice during the four-day testing period is shown in figure 11. There was no significant difference between sham and VTA-lesioned FAST mice on day 1, while the distance travelled after the ethanol injection was significantly reduced ($p < 0.05$). None of the lesions altered body weight (figure 12) or ethanol metabolism (figure 13) compared to sham-operated controls. In SLOW mice, there were no effects of lesions of any brain area on the response to ethanol (figure 10). Correlational analyses revealed no effect of the length of recovery time after surgery on the response to ethanol in FAST or SLOW mice for any lesioned brain area (data not shown).

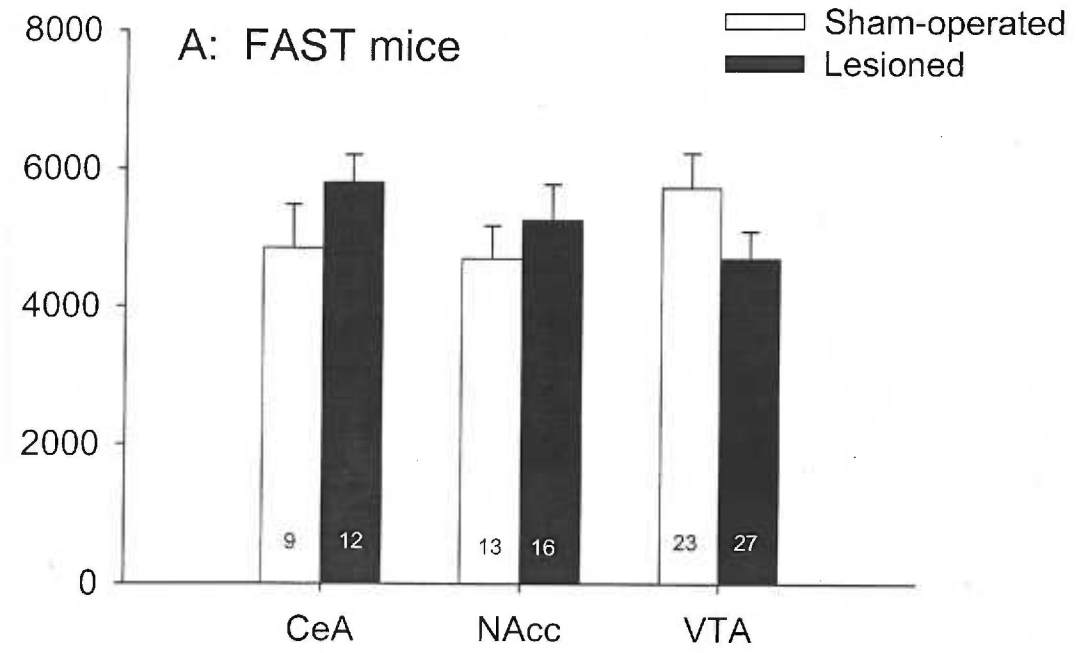
Histology

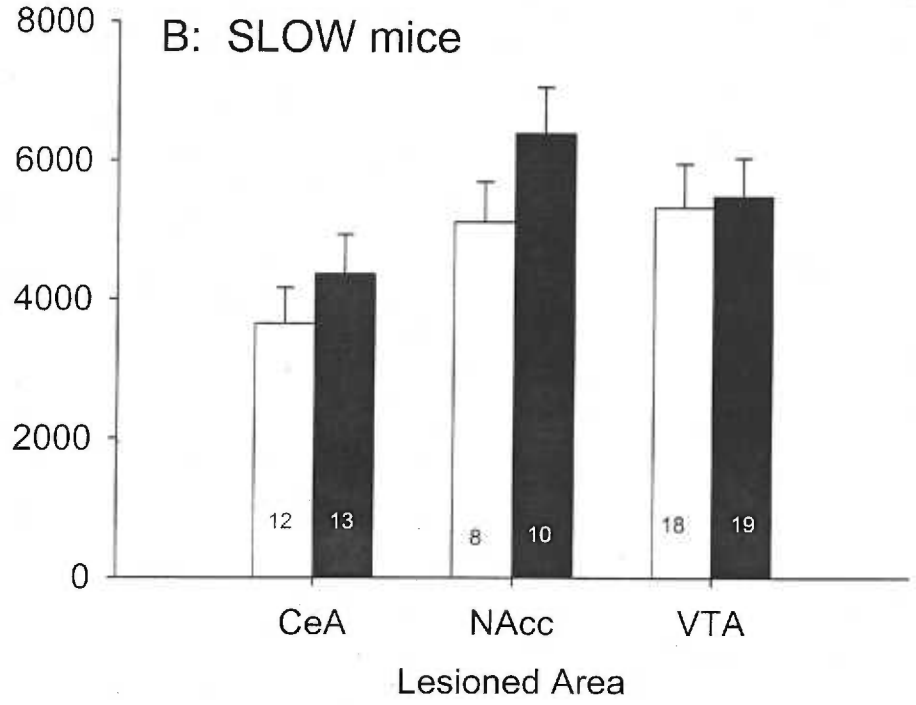
Lesions were considered "misses" if they were placed outside of the target area or damaged less than 20% of the target area. "Missed" lesions were removed from all analyses. With these criteria, overall hit rates were 78% for the CeA, 65% for the NAcc and 50% for the VTA. The majority of the misses occurred in the beginning of the experiments; surgical accuracy improved as the coordinates were adjusted based on initial histological analyses.

Examples of the location and size of the lesions are shown in figure 14. Additional examples of VTA lesions are shown in the saggital sections of figure 15. In general, lesions of the NAcc were not large enough to remove the entire NAcc, while lesions of the CeA often damaged surrounding areas as well. VTA lesions were generally restricted to anterior portions of the VTA, although some also included surrounding areas.

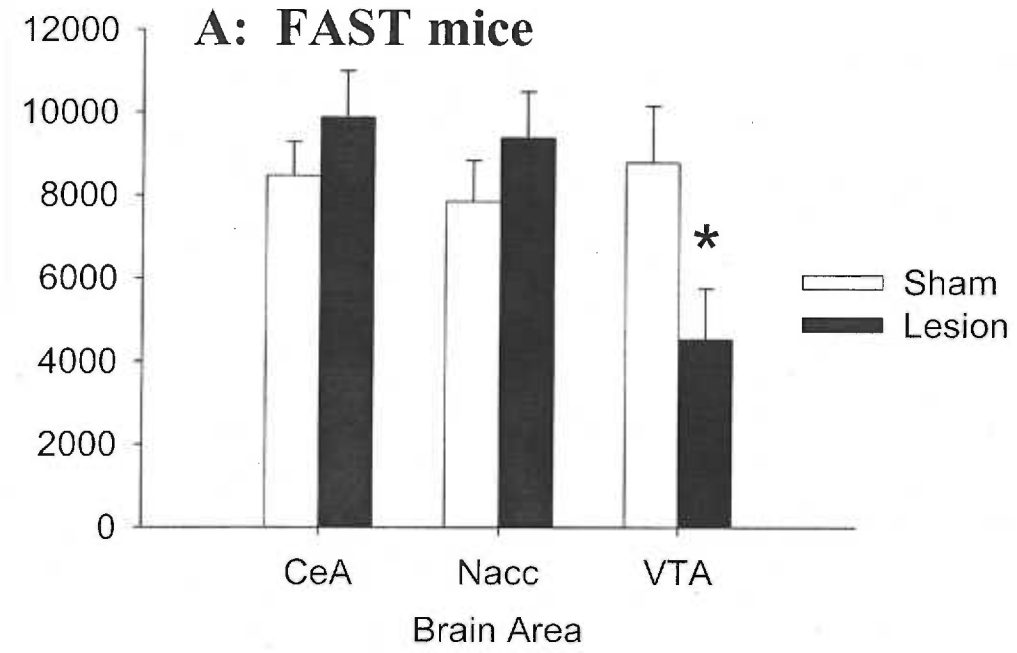
Immunohistochemistry

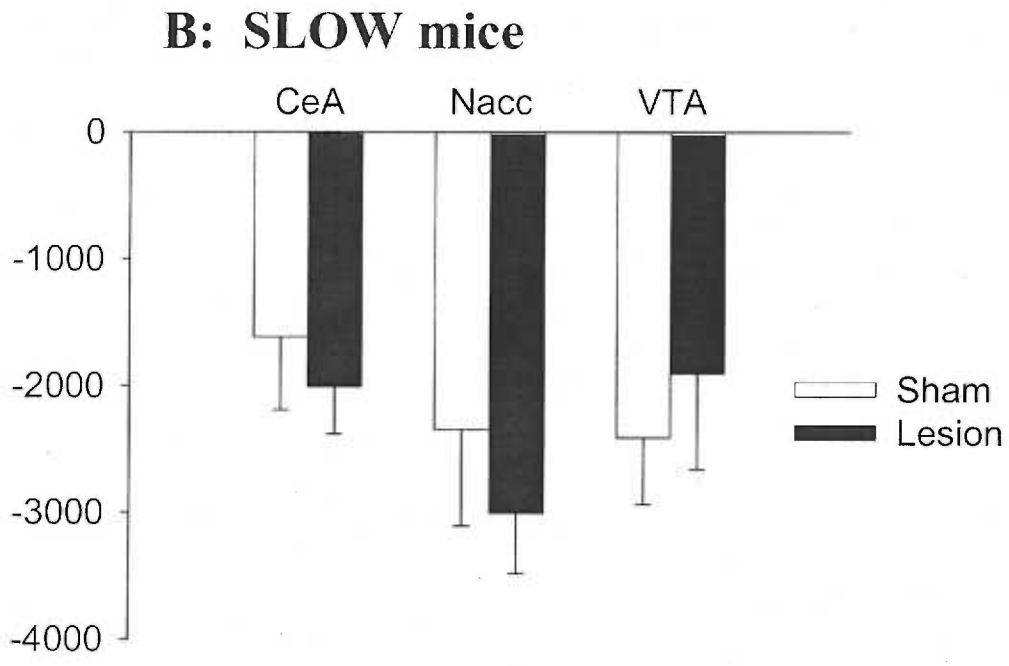
DAT-immunostained sections of the NAcc are shown in figure 16. DAT immunolabelling within the NAcc was reduced in the 6-OHDA treated mice, and there was a trend for reduced DAT staining in VTA-lesioned mice ($p = 0.08$). This suggests that VTA lesions were effective in decreasing DAT immunolabelling within the NAcc core, but not the shell.



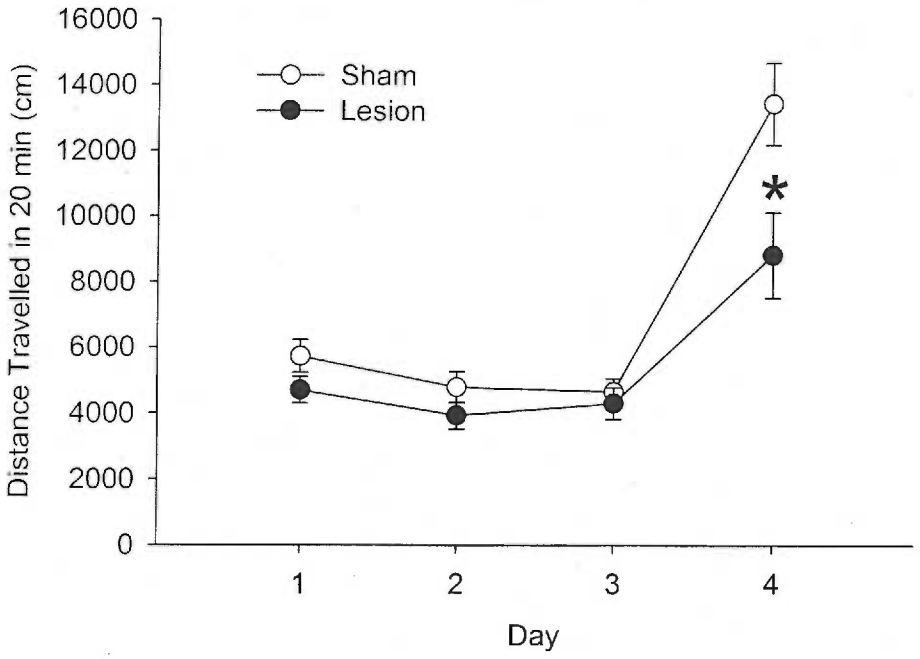


e 9: Basal activity levels on day 1 of experiment 1 were not altered by electrolytic lesions of the CeA, NAcc, or the VTA in FAST l A) or SLOW (panel B) mice. Data are represented as means ± standard error of the mean (SEM). Group sizes are indicated.





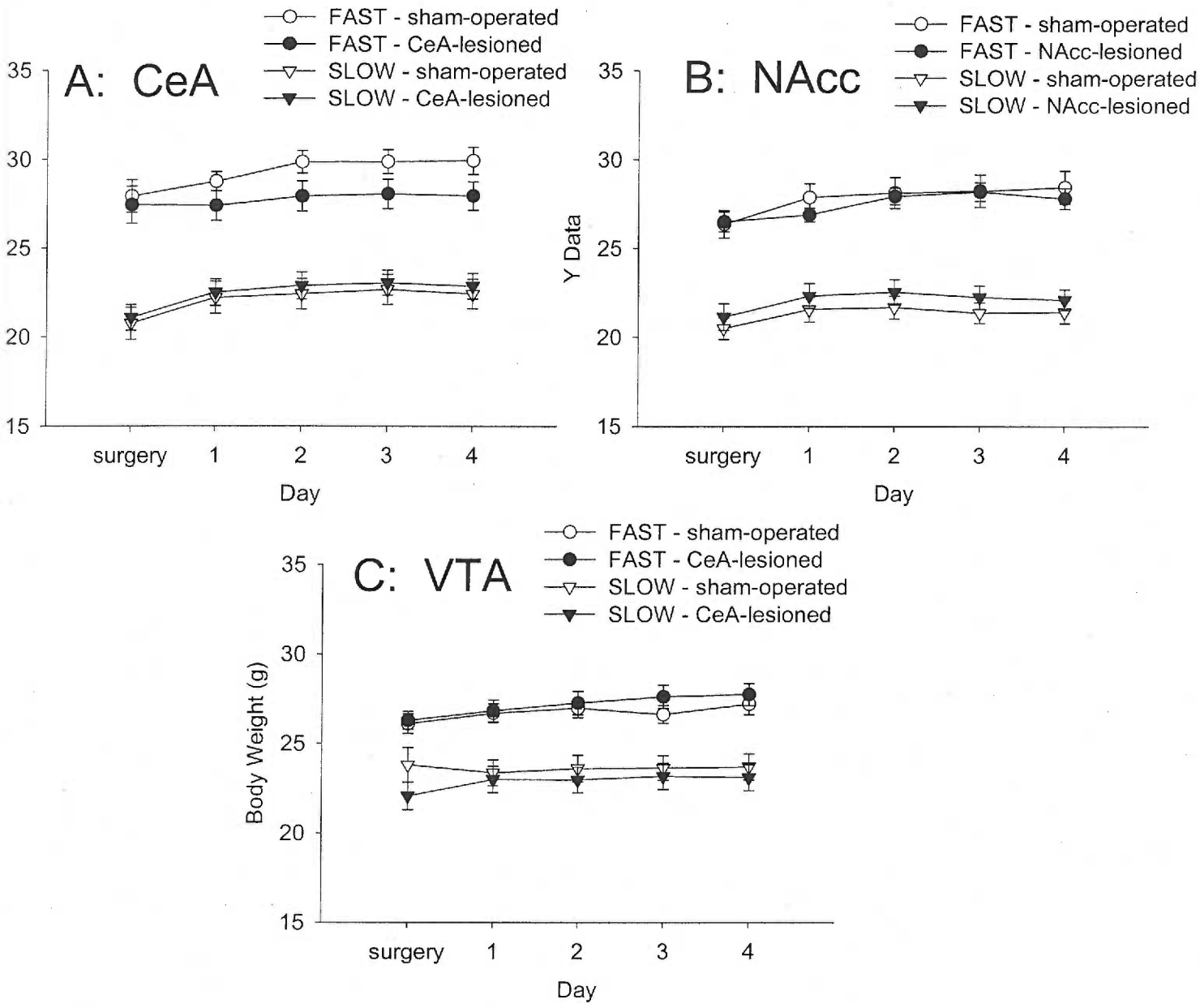
e 10: Locomotor responses to ethanol in CeA-, NAcc-, and VTA-lesioned FAST (panel A) and SLOW (panel B) mice. Data are ssed as distance travelled on day 4 (ethanol) minus distance travelled on day 3 (saline). Positive numbers reflect ethanol-induced ıotor stimulation, negative numbers reflect locomotor depression. Asterisk reflects statistical significance at $p < 0.05$. Data are sented as means ± standard error of the mean (SEM). Group sizes are as is indicated in figure 9.



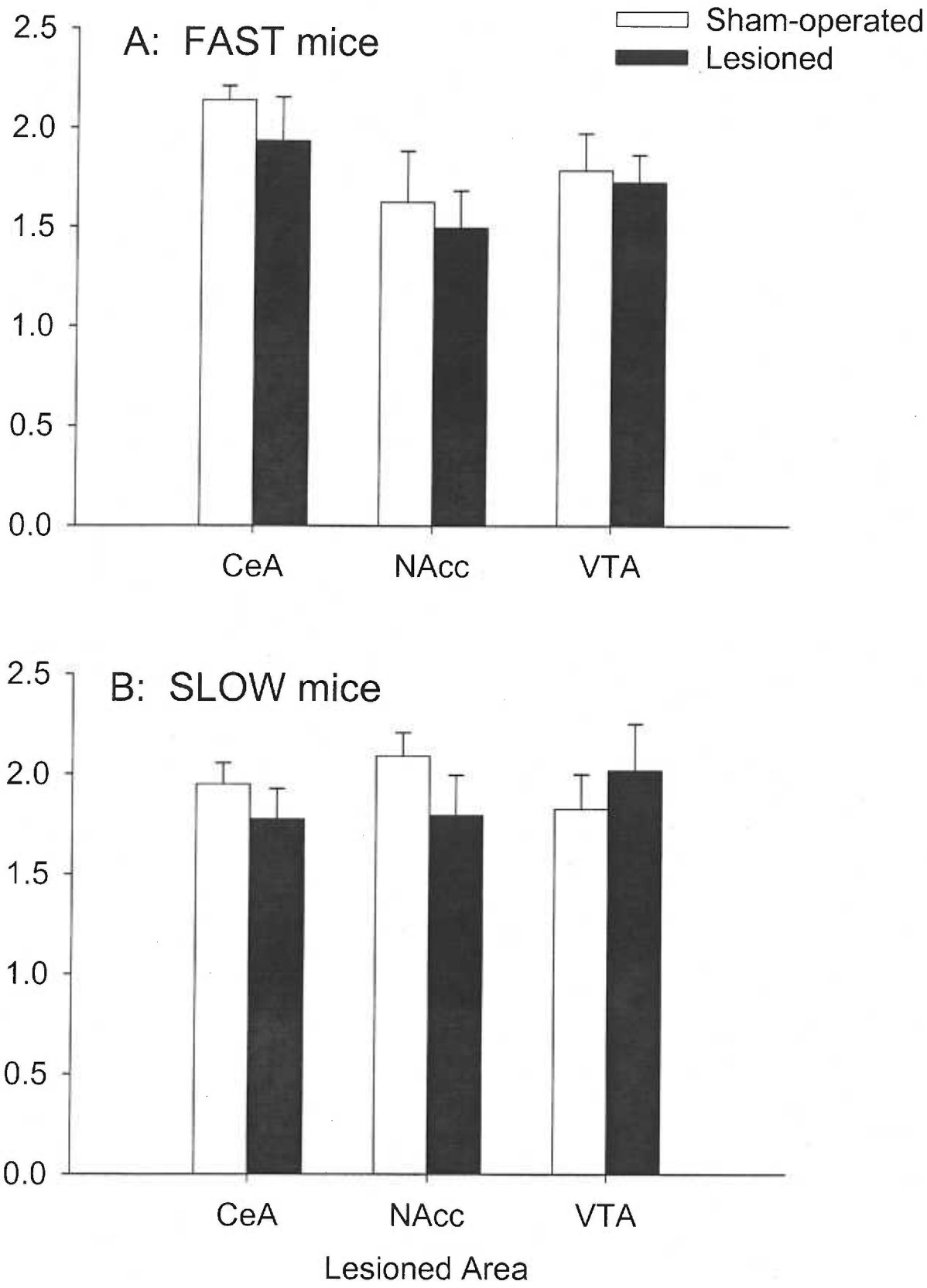
re 11: Activity levels of VTA-lesioned FAST mice over the course of the experiment. Mice were injected with i.p. saline on days 1-d with 2 g/kg ethanol on day 4. Asterisk reflects statistical significance at $p < 0.05$.

ı are represented as means ± standard error of the mean (SEM). Group sizes are 23 and 27 for sham-operated and

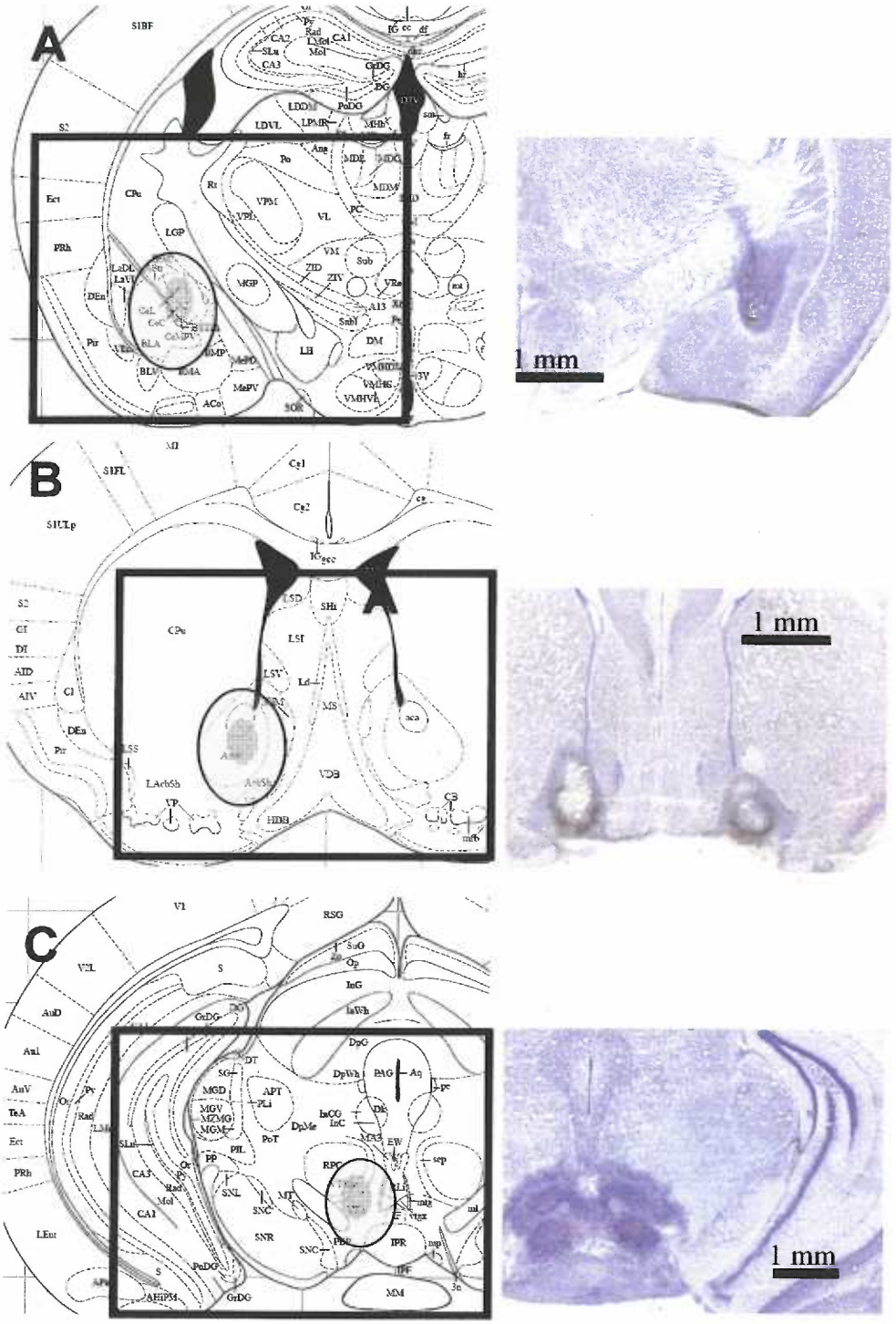
ıned mice, respectively.



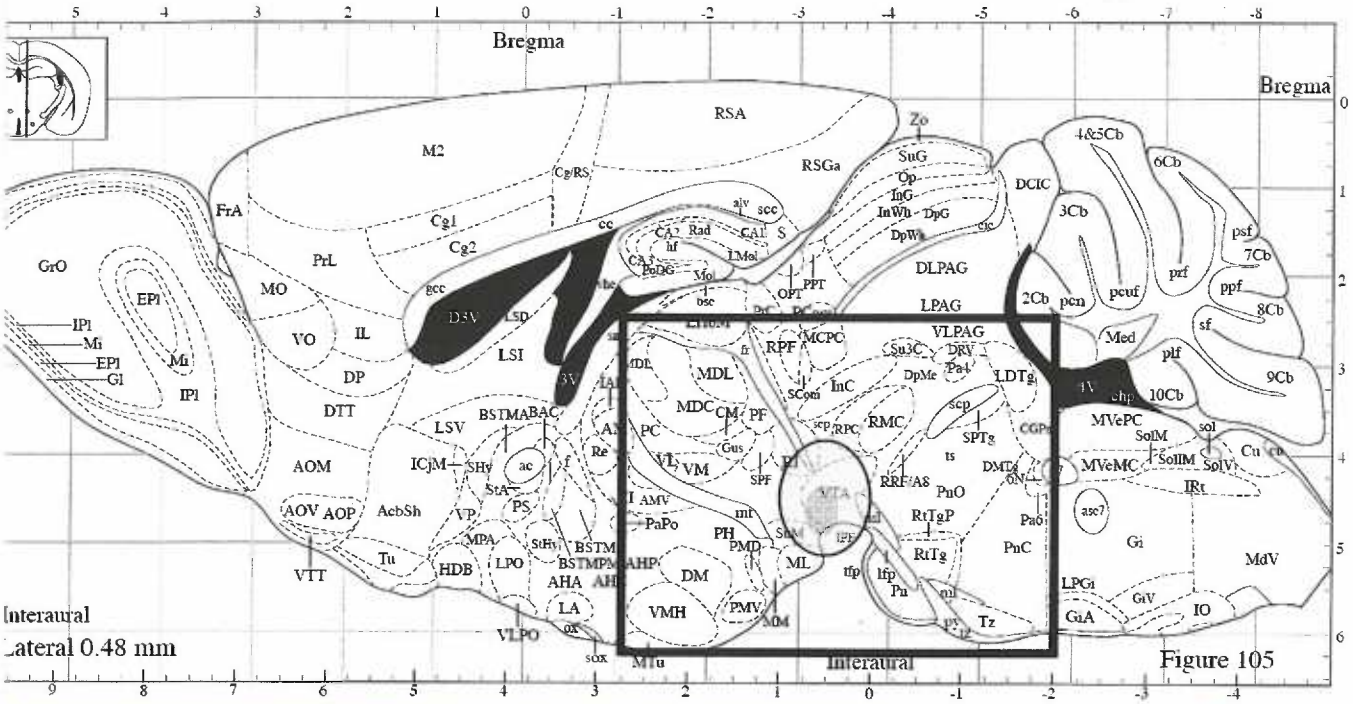
e 12: Changes in body weight of (A) CeA-lesioned, (B) NAcc-lesioned, and (C) VTA-lesioned mice over the course of the iment. Surgery occurred 1-3 weeks before behavioral testing. Data are represented as means ± standard error of the mean l). Group sizes are as is indicated in figure 9.

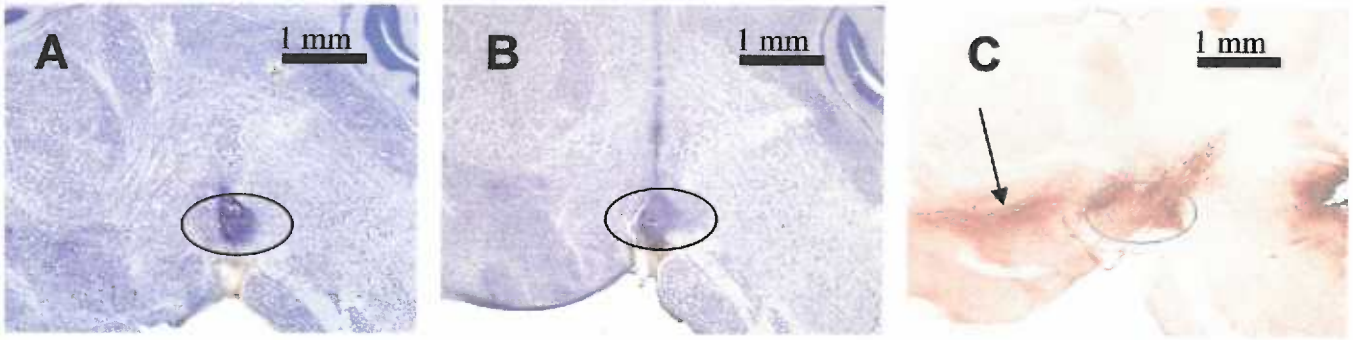


e 13: Blood ethanol content (BEC) was not altered by electrolytic lesions of the CeA, NAcc, or the VTA in FAST (panel A) or V (panel B) mice. Data are represented as means ± standard error of the mean (SEM). Group sizes are as indicated in figure 9.

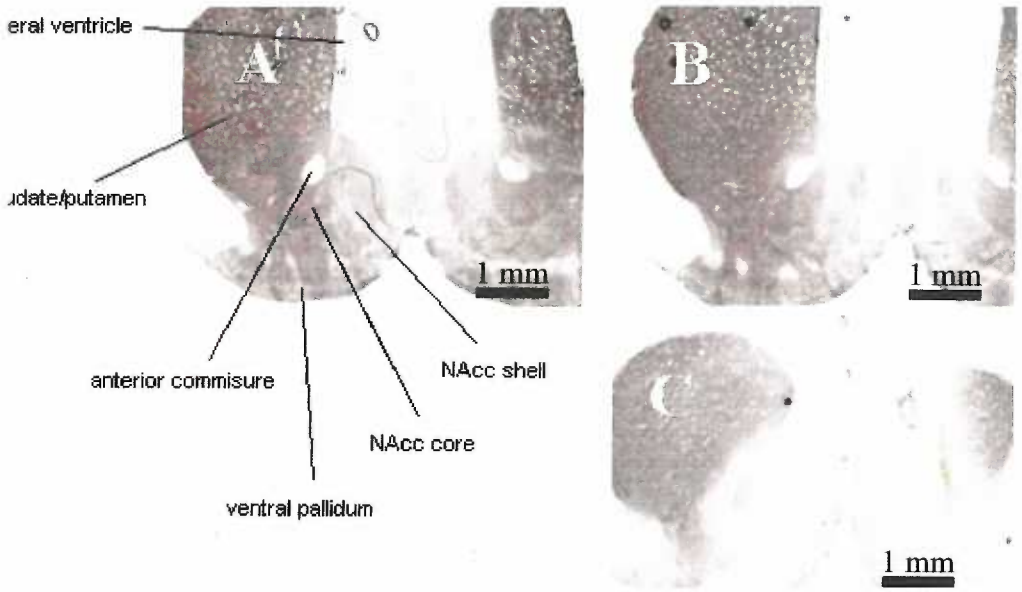


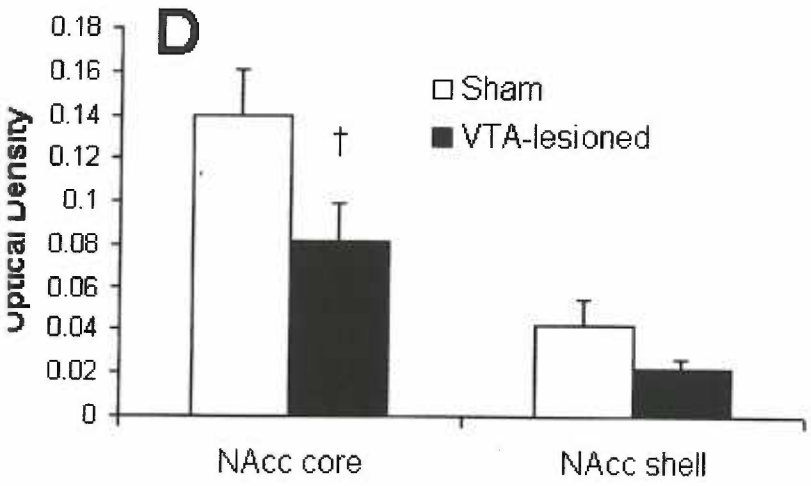
ıre 14: Examples of electrolytic brain lesions. Left panels show schematics (Paxinos and Watson, 1997) of the smallest (inner grey l) and largest (outer transparent oval) extent of the lesions. Black rectangles show the regions presented in the right panels, which w examples of actual lesions. Panels A, B, and C depict CeA, NAcc, and VTA lesions, respectively.





ure 15: Examples of VTA lesions. Top panel: Schematic of a saggital brain section through the VTA, from Paxinos and Watson 97). The small grey oval reflects the smallest lesion observed, the large transparent oval reflects the largest lesion observed. The ge rectangle indicates the area encompassed by the sections shown in panels A-C. Panels A and B depict saggital sections of typical A lesions. Ovals indicate the boundaries of the VTA. Panel C depicts a TH-immunostained section from a sham-operated mouse, wing putative dopaminergic cell bodies in the VTA and ascending dopamine fibers (arrow).





ıre 16: 6-OHDA and electrolytic VTA-lesions resulted in reduction in DAT immunostaining in the NAcc core. A) A section from a n-operated mouse, with labeled landmarks and DAT-stained subregions. B) A section from a VTA-lesioned mouse. C) A section ı a mouse that received a bilateral 6-OHDA lesion. The 6-OHDA lesioned area is revealed by the lack of DAT staining, relative to ;ham-operated mice, in areas surrounding the anterior commisure, NAcc, and nearby areas. D) Quantification of DAT ıunoreactivity indicated a trend ($p = 0.08$) for decreased DAT immunoreactivity in the NAcc core.

Experiment 2

In this experiment, one FAST and one SLOW mouse died during testing, these mice were excluded from all analyses. In several subjects, dopamine could not be detected in the dialysate samples, or there was less than a 100% increase in dopamine after the 100 mM potassium perfusion. These mice were removed from all analyses. There were 14-16 mice in each Line x Replicate category in this experiment. After collapsing across replicate (see below) there were a total of 23 FAST and 21 SLOW mice.

Activity

Basal, post-saline, and cocaine-induced locomotor activity levels are shown in figure 17A. There were basal activity differences between FAST and SLOW mice [$F(1, 40) = 10.95$; $p < 0.01$], and during the post-saline period [$F(1, 40) = 2.26$; $p < 0.05$], demonstrating that the mice had habituated to the same level before cocaine administration. Upon cocaine administration, there were large increases in activity, which were significantly higher in FAST mice compared to SLOW mice. This was supported statistically by a main effect of Line [$F(1, 40) = 5.28$; $p < 0.05$], but there was no interaction with Replicate. Perfusion of 100 mM potassium resulted in large increases in dopamine, but these increases were not different between FAST and SLOW mice.

Dopamine

Basal and post-saline dopamine dialysate concentrations were 1.19 and 1.12 nM, respectively, and did not significantly differ between FAST and SLOW mice. Therefore, data are expressed as percentages relative to the post-saline period (figure 17B). There were no differences in cocaine-induced increases between replicate 1 and 2 of FAST and

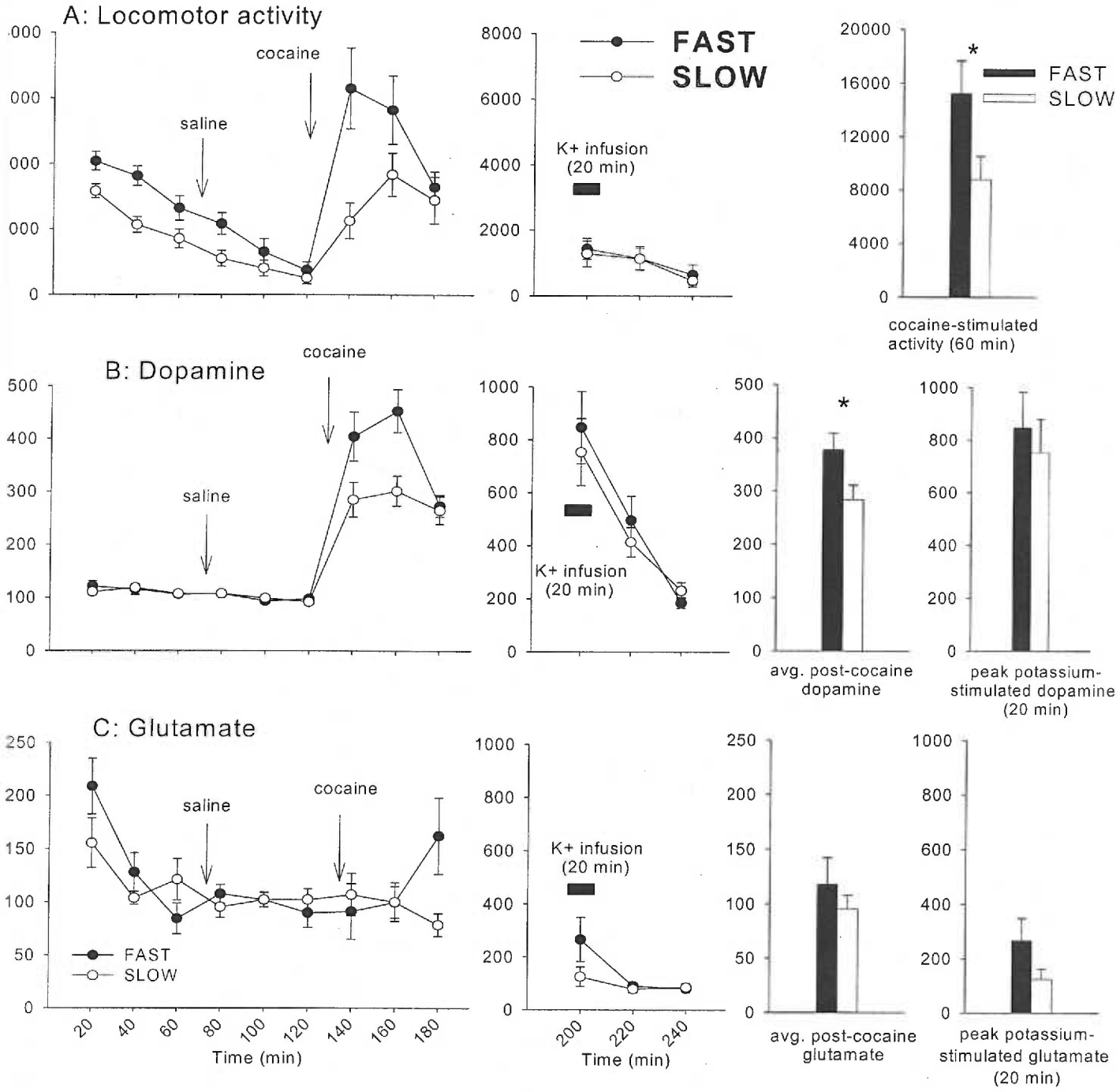
SLOW mice, so data were analyzed and presented collapsed across replicate. FAST mice showed a significantly larger dopaminergic response to cocaine, compared to SLOW mice [$F(1, 40) = 5.28$; $p < 0.05$]. There were no differences between potassium-stimulated increases in dopamine in FAST and SLOW mice.

Glutamate

Dialysate glutamate levels during experiment 2 are shown in figure 17C. There were elevated glutamate levels during the first hour of sampling (0.85 nM) that were not different between FAST and SLOW mice. Dialysate glutamate levels had stabilized by the post-saline period (0.62 nM). Cocaine caused a slight increase in glutamate, but this increase was not statistically different between the two lines. After infusion of potassium through the microdialysis probes, there was a further increase in glutamate, although this increase was not significantly different between FAST and SLOW mice.

Histology

Probe placement was 93% accurate in this study, the three mice that had probe placements outside of the NAcc had already been excluded due to lack of dialysate dopamine or potassium-stimulated increases in dopamine. Further, there were no differences in probe placement between FAST and SLOW mice (figure 18). The probes typically encompassed both the shell and the core of the NAcc, but sometimes included the striatum and the ventral pallidum as well.



e 17: Behavioral and neurochemical responses to cocaine in FAST and SLOW mice. Top, middle, and bottom panels reflect the course of the locomotor, dopaminergic, and glutamatergic responses to cocaine, respectively. Arrows indicate injections of ıe and saline at t = 60 and 120 min, respectively. The bar graphs on the right represent the cocaine- and potassium-stimulated ıres used for statistical analyses (see text for details). Asterisks reflect statistical significance at $p < 0.05$. Data are represented as s ± standard error of the mean (SEM).

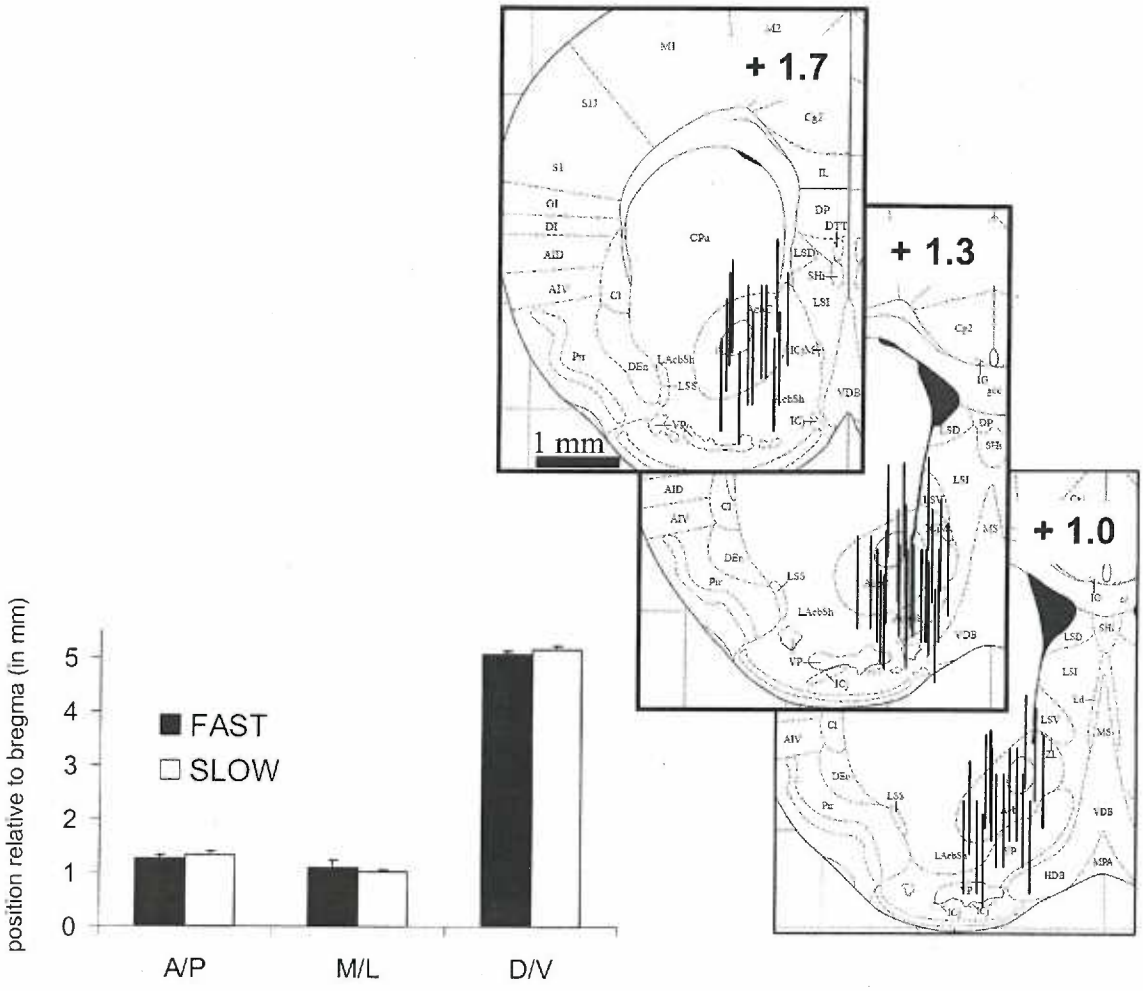


Figure 18: Location of microdialysis probes in experiment 2. Bar graph compares the mean position of the probes, in three spatial dimensions, between FAST and SLOW mice. A/P: anterior/posterior dimension; M/L medial/lateral dimension; D/V dorsal/ventral dimension. Figures on the right depict approximate locations of microdialysis probes, according to Franklin and Paxinos (1997). Numbers in the insets depict the distance relative to bregma in mm. Data are represented as means $\pm$ standard error of the mean (SEM).

Experiment 3

Activity

As in experiment 2, several mice with undetectable levels of dialysate dopamine or less than 100% increase in dopamine after perfusion of 100 mM potassium were removed from all analyses. Three FAST mice and one SLOW mouse were removed due to technical problems encountered during microdialysis and HPLC. There were 14-15 mice in each Line x Replicate category in this experiment. After collapsing across replicate (see below) there were a total of 30 FAST and 29 SLOW mice in this experiment.

Basal, post-saline, and post-ethanol activity levels are shown in figure 19A. There were no significant differences between FAST and SLOW mice in activity levels during the basal and post-saline periods. There was a larger response to ethanol in FAST mice compared to SLOW mice, as indicated by a significant effect of Line [$F(1, 57) = 43.57$; $p < 0.01$], that did not interact with replicate. Since there were was no significant difference between the two replicates of FAST and SLOW mice in ethanol-induced activity, data from the two replicates are collapsed in the figure.

Dopamine

Dialysate dopamine levels during basal, post-saline, post-ethanol, and potassium perfusion periods are shown in figure 19B. Basal and post-saline dialysate concentrations of dopamine were 1.55 and 1.47 nM; there were no significant differences in between FAST and SLOW mice. There were also no differences in dopamine levels between the lines after saline injection. Therefore, all data were expressed as percent change relative to the average of the four post-saline time points. ANOVA revealed a

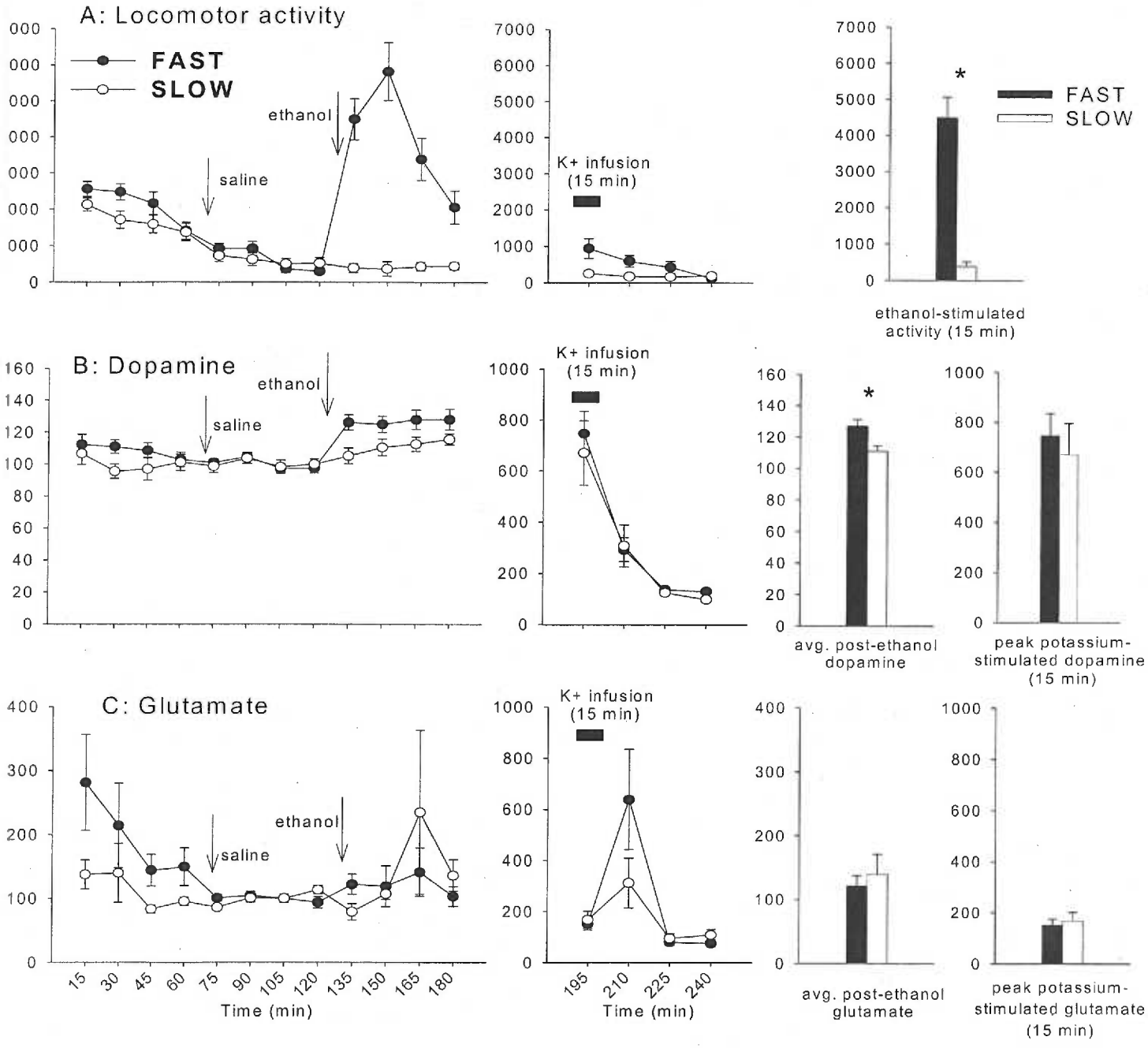
significant effect of Line [$F(1, 57) = 7.684$; $p < 0.01$], on the average of the 4 post-ethanol time points, which did not interact with replicate. These data suggest that FAST mice had a larger dopaminergic response to ethanol, compared to SLOW mice. There were no differences in potassium stimulated increases in dopamine, suggesting that the availability of releasable dopamine was not different between FAST and SLOW mice.

Glutamate

Dialysate glutamate concentrations are shown in figure 19C. Concentrations of glutamate were elevated at the beginning of the session (1.09 nM), and stabilized during the post-saline period (0.88 nM); there were no significant differences between FAST and SLOW mice during any time period. While there were potassium-stimulated increases in glutamate, there were no differences in the peak glutamate response between FAST and SLOW mice.

Histology

Probe placement was 92% accurate in this study; three of the five placements that occurred outside of the NAcc had already been removed based on a lack of dialysate dopamine or response to potassium, the other two were placed posterior to the NAcc. Data from these mice were removed from all analyses. Analysis of microdialysis probe placement was similar to that for experiment 2, which is shown in figure 18. There were no significant differences in probe placement between FAST and SLOW mice of either replicate. Probes tended to be located within both the core and shell of the NAcc. Sometimes, probes encompassed portions of the caudate-putamen or the ventral pallidum.



e 19: Behavioral and neurochemical responses to ethanol in FAST and SLOW mice. Top, middle, and bottom panels reflect the course of the locomotor, dopaminergic, and glutamatergic responses to ethanol, respectively. Arrows indicate injections of ol and saline at t = 60 and 120 min, respectively. The bar graphs on the right represent the ethanol- and potassium-stimulated ures used for statistical analyses (see text for details). Asterisks reflect statistical significance at $p < 0.05$. Data are represented as s $\pm$ standard error of the mean (SEM).

Experiment 4

Activity

We examined the effects of VTA lesions on the behavioral and neurochemical response to ethanol in FAST-2 mice. We chose to use only FAST-2 because previous data suggests that the response to ethanol is slightly larger in this mouse line, compared to FAST-1 mice (Delfs et al., 1990; Phillips et al., 1991; Porrino, 1993). Two lesioned mice had activity levels that were 3 standard deviations higher than the average values of the remaining lesioned mice. The data from these mice were removed from all analyses, resulting in final samples sizes of n=9 lesioned mice and n=11 sham-operated mice. Since ethanol has peak behavioral effects within the first 15 minutes of administration, only the 15 minutes after ethanol administration were analyzed, as in experiment 2. VTA lesions reduced the response to ethanol during this time period [$t(17) = 2.2$, $p < 0.5$], but basal and saline-induced activity were not affected (figure 20A).

Dopamine

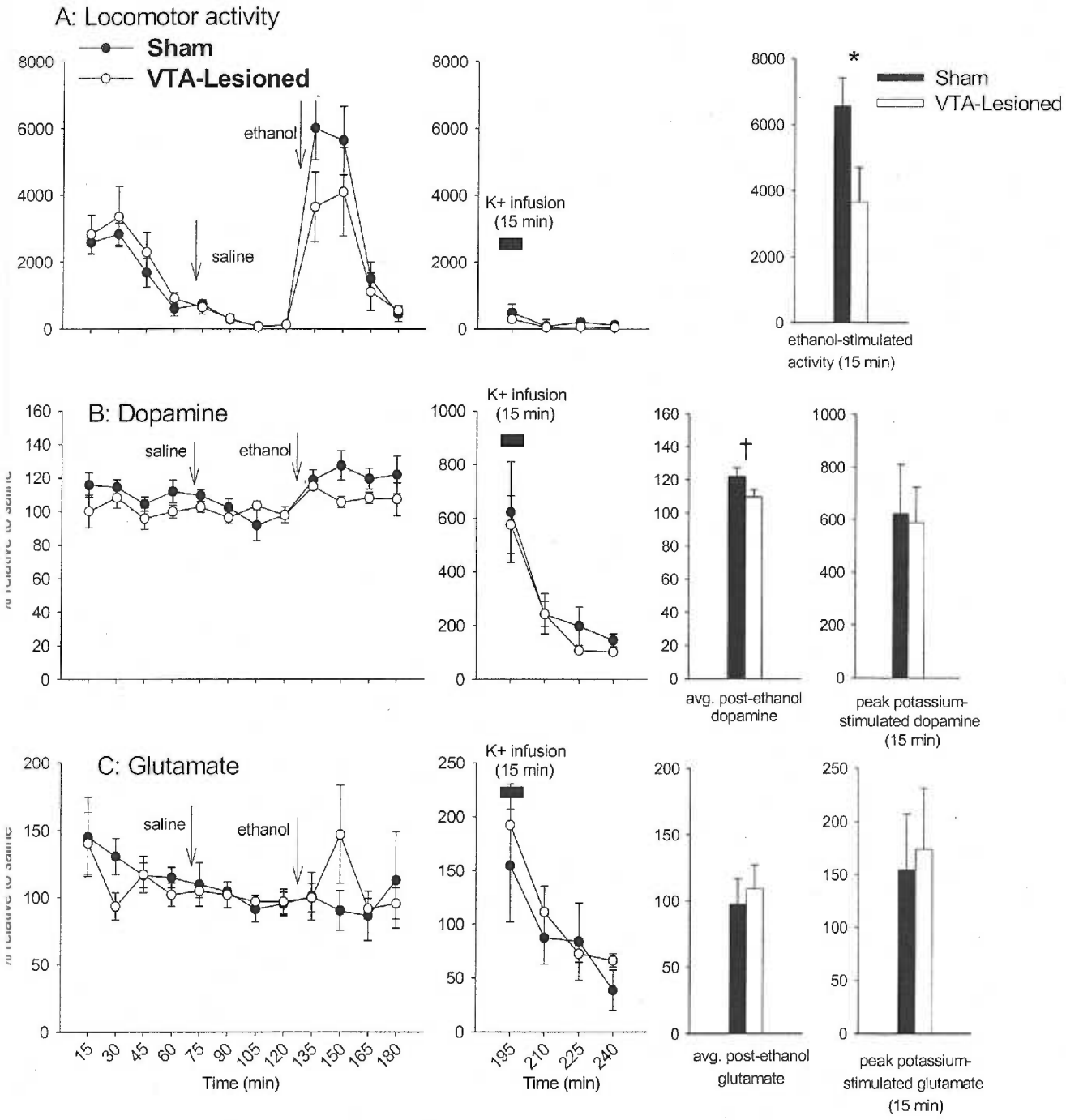
Dialysate glutamate levels were 1.47 and 1.38 nM during the basal and post-saline time periods, respectively. Similar to the activity data, VTA-lesions did not affect the basal or saline-induced dopamine levels (figure 20B). However, there was a trend indicating that VTA-lesioned mice had reduced dopaminergic responses to ethanol, compared to sham operated-mice ($p = 0.11$). There were no differences in response to potassium-stimulated increases in dopamine, suggesting partial VTA-lesions did not alter the availability of releasable vesicular dopamine.

Glutamate

Changes in glutamate levels during the experiment are shown in figure 20C. Dialysate glutamate levels were 0.72 and 0.69 nM during the basal and post-saline time periods, respectively. There was no effect of saline or ethanol administration on glutamate levels, and potassium stimulated increases in dialysate glutamate levels were not different between sham and lesioned mice.

Histology

VTA lesions were successful in 89% of the mice, while 100% of the microdialysis probes were located within the NAcc. Mice with "missed" lesions were not included in any analysis.



gure 20 (next page): Behavioral and neurochemical responses to ethanol in sham and VTA-lesioned FAST-2 mice. Top, middle, d bottom panels reflect the time-course of the locomotor, dopaminergic, and glutamatergic responses to ethanol, respectively. rows indicate injections of saline and ethanol at t = 60 and 120 min, respectively. The bar graphs on the right represent the anol- and potassium-stimulated measures used for statistical analyses (see text for details). Asterisks reflect statistical nificance at $p < 0.05$, dagger represents a statistical trend of $p = 0.11$. Data are represented as means $\pm$ standard error of the an (SEM).

Discussion

In these experiments, partial lesions of the VTA, but not lesions of the CeA or partial lesions of the NAcc, were effective in attenuating the stimulant response to ethanol in FAST mice. None of these lesions altered the locomotor depressant response to ethanol in SLOW mice. In the microdialysis studies, FAST mice were more sensitive than SLOW mice to the stimulant effects of cocaine and ethanol, which corresponded to higher levels of cocaine and ethanol-induced increases in NAcc dopamine. This shows that drug-induced increases in NAcc dopamine are genetically correlated with behavioral sensitivity to cocaine- and ethanol-induced stimulation. VTA lesions also attenuated the dopaminergic response to ethanol in FAST mice, indicating that the VTA has parallel influences on ethanol-induced locomotion and increases in NAcc dopamine. These experiments indicate that the mesolimbic dopamine system was altered during selective breeding for sensitivity to ethanol's locomotor effects, and that the VTA is a key brain region in regulating both ethanol-induced locomotor stimulation and increases in NAcc dopamine levels.

Lesion Studies

These experiments show that partial lesions of the VTA can have specific effects on drug-induced activity, without having effects on basal activity. These findings are consistent with the ability of pharmacological manipulation of the VTA to inhibit ethanol-induced activity (Boehm et al., 2002a). To our knowledge, there are no studies of ethanol-induced locomotor stimulation in VTA-lesioned mice. This may be due to technical difficulties associated with small size of the mouse brain. Some studies have created excitotoxic lesions of the striatum in C57BL/6 mice; these lesions were effective at reducing the stimulant responses to morphine

(Siegfried et al., 1982). In rats, 6-OHDA lesions of the VTA either enhanced or decreased (Breese et al., 1985; Siegfried et al., 1982) ethanol-induced locomotor sedation (Bacopoulos et al., 1979). Future studies are needed to determine whether similar lesions in mice would attenuate the response to ethanol. We also found a trend for VTA lesions to decrease DAT immunolabelling in the NAcc core, but not the NAcc shell. This provides neuroanatomical support for the effectiveness of partial VTA lesions in altering NAcc physiology as well as the response to ethanol. We also showed that DAT-immunostaining is sensitive to decreases in terminal labeling of dopamine neurons within the NAcc, because 6-OHDA lesions of the NAcc were successful at decreasing NAcc immunolabelling. The efficacy of 6-OHDA on decreasing DAT immunostaining is consistent with decreases in autoradiographic DAT binding (Louis and Clarke, 1998; Pierson et al., 2005), and TH immunostaining (Gouhier et al., 2002) after NAcc and VTA 6-OHDA lesions.

These experiments also found that NAcc lesions were ineffective at attenuating the locomotor response to ethanol. This may be because the lesions of the NAcc were not large enough to alter the response to ethanol, or that the NAcc is not involved in the response to ethanol, despite the differential increases in dopamine induced by ethanol in FAST and SLOW mice. Compared to the compact nucleus of the VTA, the projections from the VTA to the NAcc are relatively diffuse (Swanson, 1982), so it may be that ethanol-induced locomotion only requires a few intact projections, or that the required VTA-NAcc projections were not damaged by NAcc lesions in these studies. Interestingly, one study (Makanjuola and Ashcroft, 1982) found that intra-NAcc 6-OHDA attenuated the locomotor response to amphetamine, but electrolytic lesions had no effect. This suggests that the damage induced by 6-OHDA may be

more complete than that caused by an electrolytic lesion. We did not generate enough 6-OHDA lesioned mice to test the behavioral responses to ethanol, so it is not known whether these lesions would be effective in decreasing the response to ethanol. A lack of an effect of intra-NAcc 6-OHDA dopamine on ethanol stimulation in DBA/2J mice has been suggested (Hitzemann, personal communication), but it is not known whether the response in FAST mice would be altered by this treatment. Additional studies are needed to test this idea.

It may be surprising that a minor deficit in dopaminergic function results in the behavioral deficits observed here. Studies of methamphetamine-induced neurotoxicity and Parkinson's disease have suggested that normal behavioral functions remain intact despite intense degradation of dopamine signaling in the brain (Joyce et al., 1983; Stricker and Zigmond, 1976) For example, bilateral injections of 6-OHDA into the NAcc resulted in a 95% reduction in tissue dopamine content in rats (Joyce et al., 1983). While basal locomotion was reduced at three-days after the surgery, there was no difference compared to sham operated rats at one week after surgery. However, studies in humans (Volkow et al., 1997) and rodents (Wallace et al., 1999) have reported motor deficits after modest decreases in dopaminergic function, as measured by dopamine content (Wallace et al., 1999) and dopamine transporter occupancy (Volkow et al., 1997). This suggests that mild deficits in the mesolimbic dopamine system could result in the subtle alterations of behavior observed in these experiments.

Alternatively, while dopaminergic activity within the NAcc is indeed altered in FAST and SLOW mice, the specific projections to the NAcc may not be crucial for ethanol-induced stimulation. The VTA also projects to other brain regions, such as the PFC and the CeA (Fudge and Haber, 2000; Swanson, 1982). Ethanol has been shown to alter dopamine levels in the PFC

(Fadda et al., 1985), and manipulations of the PFC alter ethanol drinking (Nielsen et al., 1999; Samson and Chappell, 2001). As mentioned in the introduction, the CeA is differentially activated between FAST and SLOW mice, as measured by c-Fos expression (Demarest et al., 1999a). However, since lesions of the CeA did not have an effect on ethanol-induced locomotor activity in either FAST or SLOW mice, the differential expression observed in the CeA may either be secondary to ethanol-induced locomotion, or be related to the divergent effects of ethanol on other behaviors in FAST and SLOW mice. For example, FAST mice were less sensitive to ethanol's anxiolytic effects than were SLOW mice (Boehm et al., 2002), and show several other genetically correlated differences (Boehm et al., 2000; Boehm et al., 2002b; Shen et al., 1996). Given the role of the amygdalar complex in anxiety responses (Day et al., 2005; Holahan and White, 2004), the differential expression of c-Fos may be due to a differential sensitivity of these lines to ethanol-induced anxiolysis.

The VTA, NAcc, and surrounding areas such as the substantia nigra are involved in motivation and coordinated movement (Mogenson and Yang, 1991; Yun et al., 2004) as well as the locomotor response to novelty (Le Moal and Simon, 1991). In a series of studies by Fink and Smith (1980a; 1980b) 6-OHDA injected into the midbrain resulted in widespread damage to forebrain terminal areas. These lesions blocked the exploratory response to novel objects, and locomotor behavior in a novel testing chamber. However, these lesions did not alter activity in a familiar environment. In another study, bilateral 6-OHDA lesions of the NAcc blocked the exploratory locomotor response to a novel testing chamber (Pierce et al., 1990). Again, locomotor activity in a familiar environment was not altered by the lesion. Similar findings have been found with systemic injections of low doses of dopamine antagonists (Bardo et al., 1990;

Bardo et al., 1989). Hooks and Kalivas (1995) blocked the locomotor response to novelty by injecting a dopamine antagonist or the $GABA_B$ agonist baclofen into the NAcc or VTA, respectively. Once again, these treatments did not alter activity in a habituated environment. In FAST mice habituated to the testing chambers, microinjection of baclofen into the VTA, while altering ethanol-induced locomotion, did not alter saline-induced locomotor activity. It is unknown whether baclofen treatment in FAST mice would reduce locomotor activity in a non-habituated, or novel, environment. Together, these experiments suggest that mesolimbic dopamine is not involved in locomotion in familiar or habituated environments, but rather in response to certain stimuli such as novelty and drug exposure (Fink and Smith, 1980c). This is also consistent with studies of incentive learning, which suggest that dopamine is involved in the processing of unexpected stimuli (Schultz et al., 2003).

For these reasons, it may be somewhat surprising that there were no effects of VTA or NAcc lesions on spontaneous locomotion in a novel environment (as measured by the response to the monitors on day 1 of experiment 1). VTA-lesioned mice showed slightly lower activity levels on day 1, but this effect was not statistically significant. NAcc-lesioned mice did not show any evidence of an altered response to the chambers on day 1. VTA lesions also had no effect on activity levels during the first hour of experiment 4, when the mice were first placed directly into the activity chambers. It may be that the response to novelty involves relatively small increases in VTA function that are spared by the partial VTA lesions of this study. Meanwhile, ethanol-induced stimulation may require larger increases in dopamine that are affected by these lesions. It may also be that the initial exposure to these chambers is a qualitatively different stimulus that

does not have an identical novelty component compared to those used in the novelty preference experiments described above.

It may also be surprising that lesions of the VTA and NAcc did not alter feeding behavior, (as measured by changes in body weight after surgery). A role of these brain areas in feeding has been suggested by studies showing that microinjections of muscimol and baclofen into the VTA and NAcc induced intense feeding and drinking in rats (Arnt and Scheel-Kruger, 1979; Echo et al., 2002; Klitenick and Wirtshafter, 1988; Stratford and Kelley, 1997). However, 6-OHDA lesions of the NAcc altered feeding in food deprived but not free-feeding rats (Koob et al., 1978). Papp and Bal (1987) found that 6-OHDA lesions blocked feeding only when the mice were required to perform an operant for the food, and not during free-feeding. This suggests that VTA and NAcc lesions do not affect spontaneous feeding. Since mice were allowed access to food *ad libitum* in the current studies, this may explain why VTA and NAcc lesions did not affect body weight in these studies.

Data from initial pilot studies created VTA lesions of varying sizes by varying the current size and time applied to the electrodes. Currents larger than 0.25 mA applied for more than 5 s created large lesions that inhibited feeding behavior, resulting in severe weight loss and substantial hypoactivity. This is consistent with a role for the VTA in feeding and basal locomotion, and indicates that the use of the smaller-sized lesions may have created subtle disruptions in the VTA that did not alter its normal function, but were large enough to attenuate its response to pharmacological stimuli such as ethanol.

As discussed in the introduction, the PFC is also interconnected to the mesolimbic dopamine system; it receives dopaminergic input from the VTA (Oades and Halliday, 1987;

Swanson, 1982) and provides primarily glutamatergic input into the NAcc and the VTA (Carr and Sesack, 2000; Rossetti et al., 1998; Sesack and Pickel, 1992). While we did not include a PFC lesioned group, it is possible that VTA projections to the PFC are required for ethanol-induced stimulation. Some studies have shown ethanol-induced changes in dopamine turnover in the PFC in rats (Fadda et al., 1991), but no studies have addressed whether ethanol's effects on neurotransmission in the PFC are related to ethanol-induced locomotion, probably because microdialysis in mouse PFC is technically difficult due to lower concentrations of dopamine in the PFC than the NAcc (Feenstra et al., 2000).

An important limitation of the lesions used in these studies is that the damage occurring to the VTA may have also damaged axon fibers passing through the VTA. We chose to use electrolytic lesions in these studies because pilot studies in our laboratory suggest that excitotoxic lesions induced by drugs such as ibotenic and quinolinic acid are variable and often absent in FAST and SLOW mice (unpublished data), possibly because we used our anesthetic cocktail included ketamine, whose antagonist effects at the NMDA receptor may be neuroprotective. Interestingly, preliminary analysis of the locations of the VTA lesions in these studies has found that lesions that occurred in more anterior portions of the VTA were also effective in diminishing the locomotor stimulant response to ethanol (data not shown). More posterior lesions, on the other hand, were not as effective. The effectiveness of lesions occurring in relatively more anterior areas suggests that the VTA and its projections are involved in the effects observed here, rather than projections from nuclei posterior to the VTA. The differential effect of anterior and posterior lesions is also consistent with other studies suggesting regional heterogeneity within the VTA with regard to ethanol sensitivity (Boehm et al., 2002a; Rodd-

Henricks et al., 2000). However, the studies in this dissertation were not designed to examine the differential effects of anterior and posterior VTA lesions. Future studies using axon-sparing lesions are needed in order to determine whether these effects of the lesions are due to damage to fibers of passage through the VTA. One such approach would be to create dopamine-neuron specific lesions using drugs such as 6-OHDA.

Microdialysis Studies

These studies also demonstrated that the dopaminergic system is differentially modulated by ethanol and cocaine in FAST and SLOW mice. Since these mice are also differentially sensitive to the locomotor stimulant effects of these drugs, these data indicate that the mesolimbic dopamine system may be a common neurochemical substrate underlying the behavioral differences in drug response in FAST and SLOW mice. However, it is unknown whether cocaine and ethanol modulate this system through a common mechanism, or whether a fundamental difference exists in the neurophysiology of this system between FAST and SLOW mice. Interestingly, FAST and SLOW mice did not differ in sensitivity to the locomotor effects of cocaine until later generations of selection (Bergstrom et al., 2003). This suggests that selection for ethanol-induced locomotion has less impact on genes involved in cocaine sensitivity, compared those involved in the response to other drugs such as GABAergic compounds, or that there is relatively less genetic diversity in the genes involved in the response to cocaine.

Imperato and Di Chiara (1986) showed that gammabutyrolactone, an agent which blocks DA firing and increases in NAcc dopamine, inhibited ethanol induced dopamine increases in rats, suggesting that ethanol enhances firing rates of the dopamine neurons. Brodie et al. (1999)

have demonstrated that ethanol directly stimulates VTA neurons, but others (Boehm et al., 2002a) have suggested ethanol disinhibits VTA neurons in FAST mice via inhibition of GABA interneurons within the VTA. It is unclear whether the ethanol-stimulated dopamine increases observed in this study occurred due to direct stimulation of VTA neurons, or through local circuits within the VTA. It is also possible that ethanol acts in other brain areas that are interconnected to the VTA, thereby stimulating VTA neurons indirectly.

It is possible that selective breeding for sensitivity to ethanol altered basal NAcc dopamine levels. The current studies were not designed to test this idea. In order to assess basal neurotransmitter levels, it is appropriate to conduct a no-net-flux study (Lonnroth et al., 1987; Yim and Gonzales, 2000), in which different concentrations of a neurotransmitter are added to the aCSF. In this manner, the concentration of the neurotransmitter is measured before [NT_{in}] and after [NT_{out}] perfusion through the microdialysis. The concentration where [NT_{in}] equals [NT_{out}] reflects the basal concentration of the neurotransmitter. This procedure corrects for variation in neurotransmitter recovery that can be a result of variations in probe recovery, tissue resistance, or spontaneous oxidation of neurotransmitters (which would interfere with their detection via electrochemical methods). Since this study measured only the dialysate content of neurotransmitters, it is premature to make statements about differences in basal dopamine levels between FAST and SLOW mice, or between lesioned and sham operated mice. Instead, we expressed dopamine and glutamate levels relative to post-saline levels, which compensated for individual variations in probe recovery, and is common in microdialysis studies (Auclair et al., 2002; Dahchour et al., 1994; Ito et al., 2002; Selim and Bradberry, 1996; Yim and Gonzales, 2000; Yoshimoto et al., 2000). Therefore, we chose to use the post-saline period instead of the

preceding basal period, because dopamine levels were elevated during the basal period, possibly due to the mice's reaction to the novelty of the testing chamber (Bardo et al., 1990; Hooks and Kalivas, 1995; Rebec et al., 1997). Exposure to a novel environment has been shown to increase exploratory behavior and NAcc dopamine as measured by *in-vivo* cyclic voltammetry (Rebec et al., 1997) and microdialysis (Saigusa et al., 1999). Further, blockade of glutamatergic transmission within the VTA blocked the novelty-associated increases in NAcc dopamine, but not the exploratory response (Legault and Wise, 2001). A similar finding was found in the current studies; both locomotor activity and NAcc dopamine levels were elevated during basal time points, relative to post-saline time points.

It is interesting that FAST and SLOW mice are also differentially sensitive to the dopaminergic effects of cocaine, which acts by blocking the DAT (and other transporters as well). This suggests that cocaine may bind to the DAT with differential kinetics between FAST and SLOW mice, and that ethanol may have effects on the DAT that underlie the divergent responses to ethanol in FAST and SLOW mice as well. However, the current study is unable to determine whether increases in NAcc dopamine occurred as result of increases in dopamine release or decreases in dopamine uptake. A number of studies have suggested that ethanol increases VTA firing and subsequent release of dopamine (Brodie et al., 1999; Yim and Gonzales, 2000), while other studies have shown that ethanol can inhibit DAT function, either by inhibiting it (Lin and Chai, 1995; Tan et al., 1981), or by causing release of dopamine in a manner similar to amphetamine (Eshleman et al., 1994). The no-net-flux assay is also able to address the release vs. uptake issue, as changes in the slope of a line defined by $[NT_{in}]$ and $[NT_{out}]$ are interpreted as changes in neurotransmitter uptake. Using this technique, Yim et al.

(2000) found that ethanol increased dopamine through an increase in dopamine release, but not uptake. Future studies utilizing the no-net-flux method are needed to determine 1) if differences in basal dopamine levels exist between FAST and SLOW mice and 2) if these basal difference and/or differences in ethanol-induced increases in dopamine are due to changes in release or uptake.

Selectively breeding for increases and decreases in ethanol-induced locomotion also resulted in differences in the locomotor responses to methamphetamine (Bergstrom et al., 2003). This led us to believe that selective breeding may have led to a difference in the ability of pharmacological stimuli to increase dopamine. This idea was supported by an experiment in which FAST and SLOW mice did not differ in response to scopolamine, a muscarinic acetylcholine receptor antagonist that has stimulant effects that are independent of dopamine function within the NAcc (Drouin et al., 2002). A fundamental difference in dopaminergic function between FAST and SLOW mice would explain the differential sensitivity of these mice to a number of drugs of abuse, such as psychostimulants and morphine (Bergstrom et al., 2003), benzodiazepines and barbiturates (Palmer et al., 2002a; Phillips et al., 1992), and ketamine (Yim and Gonzales, 2000). While these studies were unable to determine whether there were differences in basal NAcc dopamine levels, data obtained from the potassium perfusion experiments indicated that the FAST and SLOW mice do not differ in the availability of releasable dopamine (Cosford et al., 1994). aCSF containing high concentrations of potassium has been used as a depolarizing stimulus to induce vesicular release of dopamine (Ripley et al., 1997), and to show that there are functional neuronal sources of dopamine within the vicinity of the microdialysis probe. We did not find any differences in potassium-stimulated increases in

dopamine between FAST and SLOW mice, which indicated that these mice do not differ in the availability of releasable dopamine within the NAcc. This suggests that differences between FAST and SLOW mice are not due to a difference in the ability of dopamine neurons in FAST and SLOW mice to produce dopamine. However, as seen in other studies (Ripley et al., 1997), we found that potassium-stimulated increases in dopamine were quite variable between individual subjects. Therefore, small but important differences in potassium-stimulated dopamine increases would have been difficult to detect.

Also of interest is the time course of ethanol's effects on locomotion and dopamine in FAST and SLOW mice. Previous studies in our laboratory have suggested that 2 g/kg (i.p.) ethanol has peak effects on locomotion within 5 min of administration, and then decreases. In the current paradigm, ethanol induced locomotion peaked within the first 15 min, but remained stable, and sometimes slightly increased, during the second 15 min period as well. This may be due to the specifics of the microdialysis experiment, in which the mice are habituated to the activity monitors on the same day when they received the ethanol injections. Habituation to the chambers usually occurs on separate days, as occurred in experiment one, as well as several other studies utilizing FAST and SLOW mice (Meyer and Phillips, 2003). These two paradigms may produce different levels of habituation to the activity chambers which may differentially affect the response to ethanol. Other studies have found that ethanol's acute (Pastor et al., 2005) and chronic (Meyer et al., 2005) effects were modulated by the degree of chamber habituation and novelty. Pastor et al. (2005) has also suggested that the involvement of the mesolimbic dopamine system can be altered by the amount of habituation. Thus, Pastor et al. (2005) found that dopamine antagonists attenuated the locomotor stimulant response to ethanol in Swiss-

Webster mice which had undergone minimal habituation, while these drugs had no effect on the ethanol-stimulation in mice that had undergone several habituation trials. Alternatively, it may be that the microdialysis equipment prevents maximal stimulation. Although the liquid swivel is counterbalanced to minimize its effect on mouse behavior, the force required to move the swivel apparatus may decrease the peak stimulant response to ethanol in FAST mice, especially when one considers that the stimulant response to ethanol is accompanied by moderate ataxia. However, the current results suggest that the VTA is involved in the response to ethanol in both a standard testing paradigm and the microdialysis paradigm used in the current experiments.

Since ethanol levels peak in the brain within 3 minutes (Ponomarev and Crabbe, 2002) and subsequently decline, the observation that ethanol-induced dopamine levels remained elevated 45-60 min after administration suggest that there is a dissociation between the time course of ethanol concentration in the brain and ethanol-induced increases in dopamine. Yim et al. (2000) have reported that, in rats, dopamine levels had returned to baseline 90 minutes after ethanol injection, while dialysate ethanol levels remained elevated. Together, these results suggest that ethanol-induced increases in dopamine are not solely related to the direct pharmacological actions of ethanol. Also, relative to other microdialysis studies in mice, it also seems that the dopaminergic response to ethanol in FAST and SLOW mice is prolonged. Previous studies have shown ethanol produces a rapid (within 10 min) increase in dopamine levels in the NAcc, which seem to correspond with the rapid increase of ethanol concentrations in the brain (Tang et al., 2003). This rapid increase in dopamine was followed by a decrease that was related to the decline in blood-ethanol levels. However, in the current studies, dopamine levels seemed to continue to increase 45-60 min after ethanol administration (figure 19B), even

though ethanol-induced stimulation had subsided substantially. Further, there were sustained increases in dopamine in SLOW mice as well, even though these mice did not show stimulation at any time point. This suggests a dissociation between ethanol-induced activity and ethanol-induced increases in dopamine levels. It may be that the neural substrates of ethanol-induced locomotor depression mask the behavioral effects of ethanol-induced dopamine in SLOW mice.

Extracellular glutamate was elevated at the beginning of the test session in these studies, which may be a response to the novelty of the testing chambers. However, there was no evidence for an acute effect of either ethanol or cocaine on NAcc glutamate levels in the current studies. A glutamatergic input into the accumbens has been demonstrated and confirmed in these studies by the ability of 100 mM potassium-containing aCSF to stimulate increases in glutamate in these mice. Some studies have reported an acute effect of ethanol on NAcc glutamate levels (Moghaddam and Bolinao, 1994; Nie et al., 1994; Yan et al., 1998), but another reported no effect (Dahchour et al., 1994). Some studies have also reported differences in ethanol-induced glutamate increases in the NAcc selectively bred HAS and LAS rats, and in rats bred for differential ethanol tolerance (Dahchour et al., 2000; Piepponen et al., 2002), which suggests that the glutamatergic responses to ethanol is genetically correlated with the behavioral sensitivity to ethanol. To our knowledge, there are no microdialysis studies measuring NAcc glutamate levels in mice after administration of ethanol or cocaine.

It is important to point out that microdialysis studies primarily measure the overflow of neurotransmitter from synapse into extrasynaptic space (Borland et al., 2005; Plock and Kloft, 2005). It is possible that important changes in synaptic glutamate are occurring, but cannot be detected with microdialysis because the glutamate increases are small enough or tightly regulated

so that glutamate does not diffuse away from the synapse and into the dialysate. A tight regulation of glutamate levels in the NAcc is likely (Drew et al., 2004), and is supported by the small increases (approximately 200% relative to post-saline levels) after potassium perfusion, compared to potassium-stimulated dopamine increases (approximately 700%). For these reasons, an acute effect of ethanol and cocaine on NAcc glutamate can not be ruled out, especially considering that, neuroanatomically, dopamine synapses often occur on glutamate terminals within the NAcc (Wang and Pickel, 2002).

It remains to be determined what neurochemical systems underlie the sedative response to ethanol in SLOW mice. These mice are also more sensitive to ethanol-induced loss of righting reflex and hypothermia (Phillips et al., 2002; Shen et al., 1996) than FAST mice, which supports that there mice are sensitive to other measures of ethanol sedation as well. As mentioned, 6-OHDA dopamine lesions of the VTA altered the sedative response in rats (Bacopoulos et al., 1979; Breese et al., 1985). We did not find either of these to be true in VTA-lesioned SLOW mice, but the lack of an increase in ethanol-induced sedation may have been because SLOW mice were already maximally sedated. Ethanol stimulated small increases in dopamine in SLOW mice, as opposed to a decrease as we hypothesized. This suggests that increases in NAcc dopamine can occur independently of locomotion. It is likely that separate neural processes govern ethanol-induced locomotor stimulation and depression, rather than having bivalent effects on a single system. The finding that VTA lesions do not have an effect on ethanol's effects in SLOW mice partially supports this. In addition, reverse selection for ethanol-induced stimulation in SLOW mice was successful, suggesting that the stimulant response in SLOW mice (possibly due to ethanol-induced increases in dopamine) were

unmasked (Phillips et al., 2002). Ethanol actions at $GABA_A$ receptors in the VP may be important. For example, intra-VP injections of betaCCT, a partial agonist of the benzodiazepine site on $GABA_A$ receptors, blocked the reinforcing effects of ethanol, and systemic injections of this drug reversed the locomotor sedation observed after an ethanol injection (June et al., 2003).

Conclusions and Future Directions

The current studies demonstrated a critical role of the VTA in ethanol-induced stimulation, and demonstrated that the sensitivity of the mesolimbic dopamine system to ethanol and cocaine was altered by selectively breeding for sensitivity to ethanol's locomotor effects. These data are also in agreement with the growing body of literature that suggest that the VTA is a common substrate for the locomotor responses to ethanol and other abused drugs, as well as being responsible for ethanol-induced dopamine increases within the NAcc.

Future studies are needed to examine further the differences between the dopaminergic systems of FAST and SLOW mice. For example, the differences in ethanol- and cocaine-stimulated increases in NAcc may be related to differences in basal dopamine levels. The current study was not designed to measure basal levels, but quantitative microdialysis studies using the no-net-flux method would enable the measurement of basal neurotransmitter levels. Further, quantitative microdialysis can be used to determine whether transient changes in extracellular dopamine are due to changes in vesicular release or in dopamine uptake and clearance (Chefer et al., 2003). Studies such as these would also provide insight into the molecular substrates of ethanol's dopamine-enhancing properties, greater ethanol-induced dopamine release in FAST mice would suggest that ethanol activates VTA neurons to a greater degree in these mice, compared to SLOW mice. On the other hand, a difference in dopamine

uptake would suggest that ethanol-induced inhibition of the dopamine transporter is larger in FAST mice, compared to SLOW mice. Ethanol's direct effects on neuronal activity and dopamine transport could also be examined using electrophysiology and studies of dopamine transporter kinetics in a synaptosomal preparation.

Future studies are also needed to examine the particular neuronal phenotype responsible for the effects seen in these studies. Since electrolytic lesions damage all cell types, a neuron-specific toxin like 6-OHDA could be used to create partial lesions of the VTA in these mice. It will also be useful to determine if lesions of VTA terminal fields other than the NAcc, such as the PFC, alter the stimulant response in FAST mice. Further, since none of the lesions were effective at altering the response to ethanol in SLOW mice, lesions of other ethanol- and locomotor-relevant nuclei, such as the Edinger-Westphal nucleus (Bachtell et al., 2002), the bed nucleus of the stria terminalis (Demarest et al., 1998), the dorsal striatum (Costa et al., 2004), and the cerebellum (Ohno and Kanazawa, 1982) should be examined in SLOW mice.

A major limitation of this dissertation is that, while ethanol differentially regulates dopamine in the NAcc of FAST and SLOW mice, and VTA lesions have parallel effects on NAcc dopamine and ethanol-induced locomotion in FAST mice, a necessary role for NAcc dopamine in ethanol-induced locomotion has not been demonstrated. Future studies will be needed to determine whether dopamine transmission specifically within the NAcc is necessary for ethanol-induced stimulation. For example, injection of catecholamine depleting agents such as reserpine directly into the NAcc may reduce the response to ethanol in FAST mice. Agents such as the $GABA_B$ agonist baclofen have also been found to block the locomotor response to

ethanol in FAST mice; it would be interesting to determine, using microdialysis, whether this is due to a blockade of ethanol's effects on NAcc dopamine.

References

Allan AM, Spuhler KP, Harris RA (1988) gamma-Aminobutyric acid-activated chloride channels: relationship to genetic differences in ethanol sensitivity. J Pharmacol Exp Ther 244: 866-870

Amalric M, Koob GF (1993) Functionally selective neurochemical afferents and efferents of the mesocorticolimbic and nigrostriatal dopamine system. Prog Brain Res 99: 209-226

Appel SB, Liu Z, McElvain MA, Brodie MS (2003) Ethanol excitation of dopaminergic ventral tegmental area neurons is blocked by quinidine. J Pharmacol Exp Ther 306: 437-446

Ariwodola OJ, Weiner JL (2004) Ethanol potentiation of GABAergic synaptic transmission may be self-limiting: role of presynaptic GABA(B) receptors. J Neurosci 24: 10679-10686

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 Sci 25: 1351-1360

Asan E (1998) The catecholaminergic innervation of the rat amygdala. Adv Anat Embryol Cell Biol 142: 1-118

Auclair A, Cotecchia S, Glowinski J, Tassin JP (2002) D-amphetamine fails to increase extracellular dopamine levels in mice lacking alpha 1b-adrenergic receptors: relationship between functional and nonfunctional dopamine release. J Neurosci 22: 9150-9154

Auclair A, Drouin C, Cotecchia S, Glowinski J, Tassin JP (2004) 5-HT2A and alpha1b-adrenergic receptors entirely mediate dopamine release, locomotor response and behavioural sensitization to opiates and psychostimulants. Eur J Neurosci 20: 3073-3084

Bachtell RK, Tsivkovskaia NO, Ryabinin AE (2002) Alcohol-induced c-Fos expression in the Edinger-Westphal nucleus: pharmacological and signal transduction mechanisms. J Pharmacol Exp Ther 302: 516-524

Bacopoulos NG, Bize I, Levine J, Van Orden LS, 3rd (1979) Modification of ethanol intoxication by dopamine agonists and antagonists. Psychopharmacology (Berl) 60: 195-201

Bain GT, Kornetsky C (1989) Ethanol oral self-administration and rewarding brain stimulation. Alcohol 6: 499-503

Baker RC, Smolen A, Smolen TN, Deitrich RA (1987) Relationship between acute ethanol-related responses in long-sleep and short-sleep mice. Alcohol Clin Exp Res 11: 574-578

Barbaccia ML, Affricano D, Trabucchi M, Purdy RH, Colombo G, Agabio R, Gessa GL (1999) Ethanol markedly increases "GABAergic" neurosteroids in alcohol-preferring rats. Eur J Pharmacol 384: R1-2

Bardo MT, Bowling SL, Pierce RC (1990) Changes in locomotion and dopamine neurotransmission following amphetamine, haloperidol, and exposure to novel environmental stimuli. Psychopharmacology (Berl) 101: 338-343

Bardo MT, Neisewander JL, Pierce RC (1989) Novelty-induced place preference behavior in rats: effects of opiate and dopaminergic drugs. Pharmacol Biochem Behav 32: 683-689

Barr AM, Zis AP, Phillips AG (2002) Repeated electroconvulsive shock attenuates the depressive-like effects of d-amphetamine withdrawal on brain reward function in rats. Psychopharmacology (Berl) 159: 196-202

Bassareo V, Tanda G, Petromilli P, Giua C, Di Chiara G (1996) Non-psychostimulant drugs of abuse and anxiogenic drugs activate with differential selectivity dopamine transmission in the nucleus accumbens and in the medial prefrontal cortex of the rat. Psychopharmacology (Berl) 124: 293-299

Becker HC, Hale RL (1991) RO15-4513 antagonizes the anxiolytic effects of ethanol in a nonshock conflict task at doses devoid of anxiogenic activity. Pharmacol Biochem Behav 39: 803-807

Becker HC, Lopez MF (2004) Increased ethanol drinking after repeated chronic ethanol exposure and withdrawal experience in C57BL/6 mice. Alcohol Clin Exp Res 28: 1829-1838

Belknap JK, Belknap ND, Berg JH, Coleman R (1977) Preabsorptive vs. postabsorptive control of ethanol intake in C57BL/6J and DBA/2J mice. Behav Genet 7: 413-425

Belknap JK, Crabbe JC, Young ER (1993) Voluntary consumption of ethanol in 15 inbred mouse strains. Psychopharmacology (Berl) 112: 503-510

Bergstrom HC, Palmer AA, Wood RD, Burkhart-Kasch S, McKinnon CS, Phillips TJ (2003) Reverse selection for differential response to the locomotor stimulant effects of ethanol provides evidence for pleiotropic genetic influence on locomotor response to other drugs of abuse. Alcohol Clin Exp Res 27: 1535-1547

Berman RF, Cannon DS (1974) The effect of prior ethanol experience on ethanol-induced saccharin aversions. Physiol Behav 12: 1041-1044

Betz H, Kuhse J, Schmieden V, Laube B, Kirsch J, Harvey RJ (1999) Structure and functions of inhibitory and excitatory glycine receptors. Ann N Y Acad Sci 868: 667-676

Bienkowski P, Kostowski W, Koros E (1999) The role of drug-paired stimuli in extinction and reinstatement of ethanol-seeking behaviour in the rat. Eur J Pharmacol 374: 315-319

Blomqvist O, Soderpalm B, Engel JA (1992) Ethanol-induced locomotor activity: involvement of central nicotinic acetylcholine receptors? Brain Res Bull 29: 173-178

Boehm SL, 2nd, Crabbe JC, Phillips TJ (2000) Sensitivity to ethanol-induced motor incoordination in FAST and SLOW selectively bred mice. Pharmacol Biochem Behav 66: 241-247

Boehm SL, 2nd, Piercy MM, Bergstrom HC, Phillips TJ (2002a) Ventral tegmental area region governs GABA(B) receptor modulation of ethanol-stimulated activity in mice. Neuroscience 115: 185-200

Boehm SL, 2nd, Ponomarev I, Jennings AW, Whiting PJ, Rosahl TW, Garrett EM, Blednov YA, Harris RA (2004) gamma-Aminobutyric acid A receptor subunit mutant mice: new perspectives on alcohol actions. Biochem Pharmacol 68: 1581-1602

Boehm SL, 2nd, Reed CL, McKinnon CS, Phillips TJ (2002b) Shared genes influence sensitivity to the effects of ethanol on locomotor and anxiety-like behaviors, and the stress axis. Psychopharmacology (Berl) 161: 54-63

Boileau I, Assaad JM, Pihl RO, Benkelfat C, Leyton M, Diksic M, Tremblay RE, Dagher A (2003) Alcohol promotes dopamine release in the human nucleus accumbens. Synapse 49: 226-231

Borland LM, Shi G, Yang H, Michael AC (2005) Voltammetric study of extracellular dopamine near microdialysis probes acutely implanted in the striatum of the anesthetized rat. J Neurosci Methods 146: 149-158

Bossert JM, Franklin KB (2003) Reinforcing versus anticonvulsant drugs: effects on intracranial self-stimulation rate-frequency M50 indices. Behav Brain Res 144: 243-247

Bowers BJ, McClure-Begley TD, Keller JJ, Paylor R, Collins AC, Wehner JM (2005) Deletion of the alpha7 nicotinic receptor subunit gene results in increased sensitivity to several behavioral effects produced by alcohol. Alcohol Clin Exp Res 29: 295-302

Brady AM, Glick SD, O'Donnell P (2005) Selective disruption of nucleus accumbens gating mechanisms in rats behaviorally sensitized to methamphetamine. J Neurosci 25: 6687-6695

Breese GR, Coyle S, Frye GD, Mueller RA (1985) Effects of TRH, ethanol, and TRH-ethanol combination on activity in rats with altered monoamine content. Pharmacol Biochem Behav 22: 1013-1018

Broadbent J, Muccino KJ, Cunningham CL (2002) Ethanol-induced conditioned taste aversion in 15 inbred mouse strains. Behav Neurosci 116: 138-148

Brodie MS, Appel SB (2000) Dopaminergic neurons in the ventral tegmental area of C57BL/6J and DBA/2J mice differ in sensitivity to ethanol excitation. Alcohol Clin Exp Res 24: 1120-1124

Brodie MS, Pesold C, Appel SB (1999) Ethanol directly excites dopaminergic ventral tegmental area reward neurons. Alcohol Clin Exp Res 23: 1848-1852

Brose N, O'Neill RD, Boutelle MG, Fillenz M (1988) Dopamine in the basal ganglia and benzodiazepine-induced sedation. Neuropharmacology 27: 589-595

Campbell AD, McBride WJ (1995) Serotonin-3 receptor and ethanol-stimulated dopamine release in the nucleus accumbens. Pharmacol Biochem Behav 51: 835-842

Cappell H, LeBlanc AE, Endrenyi L (1973) Aversive conditioning by psychoactive drugs: effects of morphine, alcohol and chlordiazepoxide. Psychopharmacologia 29: 239-246

Carelli RM, Wightman RM (2004) Functional microcircuitry in the accumbens underlying drug addiction: insights from real-time signaling during behavior. Curr Opin Neurobiol 14: 763-768

Carlezon WA, Jr., Wise RA (1996) Rewarding actions of phencyclidine and related drugs in nucleus accumbens shell and frontal cortex. J Neurosci 16: 3112-3122

Carr DB, Sesack SR (2000) Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons. J Neurosci 20: 3864-3873

Cassens GP, Mills AW (1973) Lithium and amphetamine: opposite effects on threshold of intracranial reinforcement. Psychopharmacologia 30: 283-290

Chefer VI, Zakharova I, Shippenberg TS (2003) Enhanced responsiveness to novelty and cocaine is associated with decreased basal dopamine uptake and release in the nucleus accumbens: quantitative microdialysis in rats under transient conditions. J Neurosci 23: 3076-3084

Chester JA, Cunningham CL (1999) GABA(A) receptors modulate ethanol-induced conditioned place preference and taste aversion in mice. Psychopharmacology (Berl) 144: 363-372

Church AC, Fuller JL, Dann L (1979) Alcohol intake in selected lines of mice: importance of sex and genotype. J Comp Physiol Psychol 93: 242-246

Churchill L, Kalivas PW (1994) A topographically organized gamma-aminobutyric acid projection from the ventral pallidum to the nucleus accumbens in the rat. J Comp Neurol 345: 579-595

Chutuape MA, de Wit H (1994) Relationship between subjective effects and drug preferences: ethanol and diazepam. Drug Alcohol Depend 34: 243-251

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

Colombo G, Addolorato G, Agabio R, Carai MA, Pibiri F, Serra S, Vacca G, Gessa GL (2004) Role of GABA(B) receptor in alcohol dependence: reducing effect of baclofen on alcohol intake and alcohol motivational properties in rats and amelioration of alcohol withdrawal syndrome and alcohol craving in human alcoholics. Neurotox Res 6: 403-414

Cosford RJ, Parsons LH, Justice JB, Jr. (1994) Effect of tetrodotoxin and potassium infusion on microdialysis extraction fraction and extracellular dopamine in the nucleus accumbens. Neurosci Lett 178: 175-178

Costa RM, Cohen D, Nicolelis MA (2004) Differential corticostriatal plasticity during fast and slow motor skill learning in mice. Curr Biol 14: 1124-1134

Crabbe JC (1983) Sensitivity to ethanol in inbred mice: genotypic correlations among several behavioral responses. Behav Neurosci 97: 280-289

Crabbe JC, Gallaher ES, Phillips TJ, Belknap JK (1994) Genetic determinants of sensitivity to ethanol in inbred mice. Behav Neurosci 108: 186-195

Crabbe JC, Phillips TJ, Cunningham CL, Belknap JK (1992) Genetic determinants of ethanol reinforcement. Ann N Y Acad Sci 654: 302-310

Crabbe JC, Young ER, Deutsch CM, Tam BR, Kosobud A (1987) Mice genetically selected for differences in open-field activity after ethanol. Pharmacol Biochem Behav 27: 577-581

Cunningham CL (1995) Localization of genes influencing ethanol-induced conditioned place preference and locomotor activity in BXD recombinant inbred mice. Psychopharmacology (Berl) 120: 28-41

Cunningham CL, Clemans JM, Fidler TL (2002) Injection timing determines whether intragastric ethanol produces conditioned place preference or aversion in mice. Pharmacol Biochem Behav 72: 659-668

Cunningham CL, Fidler TL, Hill KG (2000) Animal models of alcohol's motivational effects. Alcohol Res Health 24: 85-92

Cunningham CL, Malott DH, Dickinson SD, Risinger FO (1992a) Haloperidol does not alter expression of ethanol-induced conditioned place preference. Behav Brain Res 50: 1-5

Cunningham CL, Niehus DR, Malott DH, Prather LK (1992b) Genetic differences in the rewarding and activating effects of morphine and ethanol. Psychopharmacology (Berl) 107: 385-393

D'Mello GD, Stolerman IP, Booth DA, Pilcher CW (1977) Factors influencing flavour aversions conditioned with amphetamine in rats. Pharmacol Biochem Behav 7: 185-190

Da Silva GE, Vendruscolo LF, Takahashi RN (2005) Effects of ethanol on locomotor and anxiety-like behaviors and the acquisition of ethanol intake in Lewis and spontaneously hypertensive rats. Life Sci 77: 693-706

Dahchour A, Hoffman A, Deitrich R, de Witte P (2000) Effects of ethanol on extracellular amino acid levels in high-and low-alcohol sensitive rats: a microdialysis study. Alcohol Alcohol 35: 548-553

Dahchour A, Quertemont E, De Witte P (1994) Acute ethanol increases taurine but neither glutamate nor GABA in the nucleus accumbens of male rats: a microdialysis study. Alcohol Alcohol 29: 485-487

Daniell LC, Phillips TJ (1994) Differences in ethanol sensitivity of brain NMDA receptors of long-sleep and short-sleep mice. Alcohol Clin Exp Res 18: 1482-1490

Daoust M, Saligaut C, Lhuintre JP, Moore N, Flipo JL, Boismare F (1987) GABA transmission, but not benzodiazepine receptor stimulation, modulates ethanol intake by rats. Alcohol 4: 469-472

Darracq L, Drouin C, Blanc G, Glowinski J, Tassin JP (2001) Stimulation of metabotropic but not ionotropic glutamatergic receptors in the nucleus accumbens is required for the D-amphetamine-induced release of functional dopamine. Neuroscience 103: 395-403

Davidson D, Hutchison K, Dagon C, Swift R (2002) Assessing the stimulant effects of alcohol in humans. Pharmacol Biochem Behav 72: 151-156

Day HE, Nebel S, Sasse S, Campeau S (2005) Inhibition of the central extended amygdala by loud noise and restraint stress. Eur J Neurosci 21: 441-454

de Wit H, Svenson J, York A (1999) Non-specific effect of naltrexone on ethanol consumption in social drinkers. Psychopharmacology (Berl) 146: 33-41

de Wit H, Uhlenhuth EH, Pierri J, Johanson CE (1987) Individual differences in behavioral and subjective responses to alcohol. Alcohol Clin Exp Res 11: 52-59

Deckel AW, Vavrousek-Jakuba E, Shoemaker WJ (1995) Prefrontal levels of 5-HIAA, but not dopamine, predict alcohol consumption in male Wistar rats following 6-OHDA lesions. Alcohol 12: 563-568

Delfs JM, Schreiber L, Kelley AE (1990) Microinjection of cocaine into the nucleus accumbens elicits locomotor activation in the rat. J Neurosci 10: 303-310

Demarest K, Hitzemann B, Mahjubi E, McCaughran J, Jr., Hitzemann R (1998) Further evidence that the central nucleus of the amygdala is associated with the ethanol-induced locomotor response. Alcohol Clin Exp Res 22: 1531-1537

Demarest K, Hitzemann B, Phillips T, Hitzemann R (1999a) Ethanol-induced expression of c-Fos differentiates the FAST and SLOW selected lines of mice. Alcohol Clin Exp Res 23: 87-95

Demarest K, McCaughran J, Jr., Mahjubi E, Cipp L, Hitzemann R (1999b) Identification of an acute ethanol response quantitative trait locus on mouse chromosome 2. J Neurosci 19: 549-561

Di Chiara G (2002) Nucleus accumbens shell and core dopamine: differential role in behavior and addiction. Behav Brain Res 137: 75-114

Di Chiara G, Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci U S A 85: 5274-5278

Dildy JE, Leslie SW (1989) Ethanol inhibits NMDA-induced increases in free intracellular Ca2+ in dissociated brain cells. Brain Res 499: 383-387

Donzanti BA, Uretsky NJ (1983) Effects of excitatory amino acids on locomotor activity after bilateral microinjection into the rat nucleus accumbens: possible dependence on dopaminergic mechanisms. Neuropharmacology 22: 971-981

Drew KL, Pehek EA, Rasley BT, Ma YL, Green TK (2004) Sampling glutamate and GABA with microdialysis: suggestions on how to get the dialysis membrane closer to the synapse. J Neurosci Methods 140: 127-131

Drouin C, Darracq L, Trovero F, Blanc G, Glowinski J, Cotecchia S, Tassin JP (2002) Alpha1b-adrenergic receptors control locomotor and rewarding effects of psychostimulants and opiates. J Neurosci 22: 2873-2884

Dudek BC, Phillips TJ (1983) Locomotor stimulant and intoxicant properties of methanol, ethanol, tertiary butanol and pentobarbital in Long-Sleep and Short-Sleep mice. Subst Alcohol Actions Misuse 4: 31-36

Due DL, Huettel SA, Hall WG, Rubin DC (2002) Activation in mesolimbic and visuospatial neural circuits elicited by smoking cues: evidence from functional magnetic resonance imaging. Am J Psychiatry 159: 954-960

Duka T, Tasker R, Stephens DN (1998) Alcohol choice and outcome expectancies in social drinkers. Behav Pharmacol 9: 643-653

Duvauchelle CL, Ikegami A, Castaneda E (2000) Conditioned increases in behavioral activity and accumbens dopamine levels produced by intravenous cocaine. Behav Neurosci 114: 1156-1166

Echo JA, Lamonte N, Ackerman TF, Bodnar RJ (2002) Alterations in food intake elicited by GABA and opioid agonists and antagonists administered into the ventral tegmental area region of rats. Physiol Behav 76: 107-116

Eckardt MJ (1975) The role of orosensory stimuli from ethanol and blood-alcohol levels in producing conditioned taste aversion in the rat. Psychopharmacologia 44: 267-271

El-Fakahany EF, Miller ER, Abbassy MA, Eldefrawi AT, Eldefrawi ME (1983) Alcohol modulation of drug binding to the channel sites of the nicotinic acetylcholine receptor. J Pharmacol Exp Ther 224: 289-296

Enggasser JL, de Wit H (2001) Haloperidol reduces stimulant and reinforcing effects of ethanol in social drinkers. Alcohol Clin Exp Res 25: 1448-1456

Erickson CK, Kochhar A (1985) An animal model for low dose ethanol-induced locomotor stimulation: behavioral characteristics. Alcohol Clin Exp Res 9: 310-314

Erwin VG, Jones BC (1993) Genetic correlations among ethanol-related behaviors and neurotensin receptors in long sleep (LS) x short sleep (SS) recombinant inbred strains of mice. Behav Genet 23: 191-196

Escher T, Mittleman G (2004) Effects of ethanol and GABAB drugs on working memory in C57BL/6J and DBA/2J mice. Psychopharmacology (Berl) 176: 166-174

Eshleman AJ, Henningsen RA, Neve KA, Janowsky A (1994) Release of dopamine via the human transporter. Mol Pharmacol 45: 312-316

Everitt BJ, Dickinson A, Robbins TW (2001) The neuropsychological basis of addictive behaviour. Brain Res Brain Res Rev 36: 129-138

Fadda F, Colombo G, Gessa GL (1991) Genetic sensitivity to effect of ethanol on dopaminergic system in alcohol preferring rats. Alcohol Alcohol Suppl 1: 439-442

Fadda F, Mosca E, Meloni R, Gessa GL (1985) Ethanol-stress interaction on dopamine metabolism in the medial prefrontal cortex. Alcohol Drug Res 6: 449-454

Feenstra MG, Botterblom MH, Mastenbroek S (2000) Dopamine and noradrenaline efflux in the prefrontal cortex in the light and dark period: effects of novelty and handling and comparison to the nucleus accumbens. Neuroscience 100: 741-748

File SE, Pellow S (1985) No cross-tolerance between the stimulatory and depressant actions of benzodiazepines in mice. Behav Brain Res 17: 1-7

Fink JS, Smith GP (1980a) Mesolimbic and mesocortical dopaminergic neurons are necessary for normal exploratory behavior in rats. Neurosci Lett 17: 61-65

Fink JS, Smith GP (1980b) Mesolimbicocortical dopamine terminal fields are necessary for normal locomotor and investigatory exploration in rats. Brain Res 199: 359-384

Fink JS, Smith GP (1980c) Relationships between selective denervation of dopamine terminal fields in the anterior forebrain and behavioral responses to amphetamine and apomorphine. Brain Res 201: 107-127

Finlay JM, Damsma G, Fibiger HC (1992) Benzodiazepine-induced decreases in extracellular concentrations of dopamine in the nucleus accumbens after acute and repeated administration. Psychopharmacology (Berl) 106: 202-208

Finn DA, Sinnott RS, Ford MM, Long SL, Tanchuck MA, Phillips TJ (2004) Sex differences in the effect of ethanol injection and consumption on brain allopregnanolone levels in C57BL/6 mice. Neuroscience 123: 813-819

Franklin KBJ, Paxinos G (1997) The mouse brain in stereotaxic coordinates. Academic Press, Academic Press

French ED (1994) Phencyclidine and the midbrain dopamine system: electrophysiology and behavior. Neurotoxicol Teratol 16: 355-362

Fudge JL, Haber SN (2000) The central nucleus of the amygdala projection to dopamine subpopulations in primates. Neuroscience 97: 479-494

Gabriel KI, Cunningham CL, Finn DA (2004) Allopregnanolone does not influence ethanol-induced conditioned place preference in DBA/2J mice. Psychopharmacology (Berl) 176: 50-56

Garris PA, Kilpatrick M, Bunin MA, Michael D, Walker QD, Wightman RM (1999) Dissociation of dopamine release in the nucleus accumbens from intracranial self-stimulation. Nature 398: 67-69

Gianoulakis C (2001) Influence of the endogenous opioid system on high alcohol consumption and genetic predisposition to alcoholism. J Psychiatry Neurosci 26: 304-318

Gonzales RA, Job MO, Doyon WM (2004) The role of mesolimbic dopamine in the development and maintenance of ethanol reinforcement. Pharmacol Ther 103: 121-146

Goudie AJ, Dickins DW, Thornton EW (1978) Cocaine-induced conditioned taste aversions in rats. Pharmacol Biochem Behav 8: 757-761

Gouhier C, Chalon S, Aubert-Pouessel A, Venier-Julienne MC, Jollivet C, Benoit JP, Guilloteau D (2002) Protection of dopaminergic nigrostriatal afferents by GDNF delivered by microspheres in a rodent model of Parkinson's disease. Synapse 44: 124-131

Grahame NJ, Rodd-Henricks K, Li TK, Lumeng L (2000) Ethanol locomotor sensitization, but not tolerance correlates with selection for alcohol preference in high- and low-alcohol preferring mice. Psychopharmacology (Berl) 151: 252-260

Grant BF, Stinson FS, Harford TC (2001) Age at onset of alcohol use and DSM-IV alcohol abuse and dependence: a 12-year follow-up. J Subst Abuse 13: 493-504

Grobin AC, Matthews DB, Devaud LL, Morrow AL (1998) The role of GABA(A) receptors in the acute and chronic effects of ethanol. Psychopharmacology (Berl) 139: 2-19

Hanania T, Zahniser NR (2002) Locomotor activity induced by noncompetitive NMDA receptor antagonists versus dopamine transporter inhibitors: opposite strain differences in inbred long-sleep and short-sleep mice. Alcohol Clin Exp Res 26: 431-440

Harwood H (2000) Updating Estimates of the Economic Costs of Alcohol Abuse in the United States: Estimates, Update Methods and Data. Report prepared by the Lewin Group for the National Institute on Alcohol Abuse and Alcoholism

Hayes RJ, Gardner EL (2004) The basolateral complex of the amygdala mediates the modulation of intracranial self-stimulation threshold by drug-associated cues. Eur J Neurosci 20: 273-280

Heath AC, Madden PA, Bucholz KK, Dinwiddie SH, Slutske WS, Bierut LJ, Rohrbaugh JW, Statham DJ, Dunne MP, Whitfield JB, Martin NG (1999) Genetic differences in alcohol sensitivity and the inheritance of alcoholism risk. Psychol Med 29: 1069-1081

Heath AC, Whitfield JB, Madden PA, Bucholz KK, Dinwiddie SH, Slutske WS, Bierut LJ, Statham DB, Martin NG (2001) Towards a molecular epidemiology of alcohol

dependence: analysing the interplay of genetic and environmental risk factors. Br J Psychiatry Suppl 40: s33-40

Heinz A, Siessmeier T, Wrase J, Hermann D, Klein S, Grusser SM, Flor H, Braus DF, Buchholz HG, Grunder G, Schreckenberger M, Smolka MN, Rosch F, Mann K, Bartenstein P (2004) Correlation between dopamine D(2) receptors in the ventral striatum and central processing of alcohol cues and craving. Am J Psychiatry 161: 1783-1789

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

Hinckley C, Seebach B, Ziskind-Conhaim L (2005) Distinct roles of glycinergic and GABAergic inhibition in coordinating locomotor-like rhythms in the neonatal mouse spinal cord. Neuroscience 131: 745-758

Hitzemann B, Hitzemann R (1997) Genetics ethanol and the Fos response: a comparison of the C57BL/6J and DBA/2J inbred mouse strains. Alcohol Clin Exp Res 21: 1497-1507

Hitzemann R, Hitzemann B, Rivera S, Gatley J, Thanos P, Shou LL, Williams RW (2003) Dopamine D2 receptor binding, Drd2 expression and the number of dopamine neurons in the BXD recombinant inbred series: genetic relationships to alcohol and other drug associated phenotypes. Alcohol Clin Exp Res 27: 1-11

Hodge CW, Kelley SP, Bratt AM, Iller K, Schroeder JP, Besheer J (2004) 5-HT(3A) receptor subunit is required for 5-HT3 antagonist-induced reductions in alcohol drinking. Neuropsychopharmacology 29: 1807-1813

Holahan MR, White NM (2004) Amygdala c-Fos induction corresponds to unconditioned and conditioned aversive stimuli but not to freezing. Behav Brain Res 152: 109-120

Holdstock L, de Wit H (1998) Individual differences in the biphasic effects of ethanol. Alcohol Clin Exp Res 22: 1903-1911

Holdstock L, King AC, de Wit H (2000) Subjective and objective responses to ethanol in moderate/heavy and light social drinkers. Alcohol Clin Exp Res 24: 789-794

Holstein SE, Pastor R, Meyer PJ, Phillips TJ (2005) Naloxone does not attenuate the locomotor effects of ethanol in FAST, SLOW, or two heterogeneous stocks of mice. Psychopharmacology (Berl): 1-13

Hooks MS, Kalivas PW (1995) The role of mesoaccumbens--pallidal circuitry in novelty-induced behavioral activation. Neuroscience 64: 587-597

Hunt T, Amit Z (1987) Conditioned taste aversion induced by self-administered drugs: paradox revisited. Neurosci Biobehav Rev 11: 107-130

Ikemoto S, Panksepp J (1999) The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. Brain Res Brain Res Rev 31: 6-41

Im WB, Blakeman DP, Davis JP, Ayer DE (1990) Studies on the mechanism of interactions between anesthetic steroids and gamma-aminobutyric acidA receptors. Mol Pharmacol 37: 429-434

Imperato A, Di Chiara G (1986) Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. J Pharmacol Exp Ther 239: 219-228

Ito R, Dalley JW, Robbins TW, Everitt BJ (2002) Dopamine release in the dorsal striatum during cocaine-seeking behavior under the control of a drug-associated cue. J Neurosci 22: 6247-6253

Ito R, Robbins TW, Everitt BJ (2004) Differential control over cocaine-seeking behavior by nucleus accumbens core and shell. Nat Neurosci 7: 389-397

Joel D, Zohar O, Afek M, Hermesh H, Lerner L, Kuperman R, Gross-Isseroff R, Weizman A, Inzelberg R (2005) Impaired procedural learning in obsessive-compulsive disorder and Parkinson's disease, but not in major depressive disorder. Behav Brain Res 157: 253-263

Johnson SW, North RA (1992) Opioids excite dopamine neurons by hyperpolarization of local interneurons. J Neurosci 12: 483-488

Joyce EM, Stinus L, Iversen SD (1983) Effect of injections of 6-OHDA into either nucleus accumbens septi or frontal cortex on spontaneous and drug-induced activity. Neuropharmacology 22: 1141-1145

June HL, Foster KL, McKay PF, Seyoum R, Woods JE, Harvey SC, Eiler WJ, Grey C, Carroll MR, McCane S, Jones CM, Yin W, Mason D, Cummings R, Garcia M, Ma C, Sarma PV, Cook JM, Skolnick P (2003) The reinforcing properties of alcohol are mediated by GABA(A1) receptors in the ventral pallidum. Neuropsychopharmacology 28: 2124-2137

Kalant H, Le AD (1983) Effects of ethanol on thermoregulation. Pharmacol Ther 23: 313-364

Kalant H, LeBlanc AE, Gibbins RJ (1971) Tolerance to, and dependence on, some non-opiate psychotropic drugs. Pharmacol Rev 23: 135-191

Kalen P, Skagerberg G, Lindvall O (1988) Projections from the ventral tegmental area and mesencephalic raphe to the dorsal raphe nucleus in the rat. Evidence for a minor dopaminergic component. Exp Brain Res 73: 69-77

Kalivas PW, Churchill L, Klitenick MA (1993) GABA and enkephalin projection from the nucleus accumbens and ventral pallidum to the ventral tegmental area. Neuroscience 57: 1047-1060

Kalivas PW, Duffy P, Eberhardt H (1990) Modulation of A10 dopamine neurons by gamma-aminobutyric acid agonists. J Pharmacol Exp Ther 253: 858-866

Kelly PH, Seviour PW, Iversen SD (1975) Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res 94: 507-522

Khanna JM, Morato GS, Chau A, Shah G, Kalant H (1994) Effect of NMDA antagonists on rapid and chronic tolerance to ethanol: importance of intoxicated practice. Pharmacol Biochem Behav 48: 755-763

Kiianmaa K (1978) Decreased intoxicating effect of ethanol in rats after 6-hydroxydopamine-induced degeneration of ascending dopamine pathways. Pharmacol Biochem Behav 9: 391-393

King AC, Houle T, de Wit H, Holdstock L, Schuster A (2002) Biphasic alcohol response differs in heavy versus light drinkers. Alcohol Clin Exp Res 26: 827-835

Klitenick MA, DeWitte P, Kalivas PW (1992) Regulation of somatodendritic dopamine release in the ventral tegmental area by opioids and GABA: an in vivo microdialysis study. J Neurosci 12: 2623-2632

Klitenick MA, Wirtshafter D (1988) Comparative studies of the ingestive behaviors produced by microinjections of muscimol into the midbrain raphe nuclei of the ventral tegmental area of the rat. Life Sci 42: 775-782

Kobayashi T, Ikeda K, Kojima H, Niki H, Yano R, Yoshioka T, Kumanishi T (1999) Ethanol opens G-protein-activated inwardly rectifying K+ channels. Nat Neurosci 2: 1091-1097

Koek W, Colpaert FC, Woods JH, Kamenka JM (1989) The phencyclidine (PCP) analog N-[1-(2-benzo(B)thiophenyl) cyclohexyl]piperidine shares cocaine-like but not other characteristic behavioral effects with PCP, ketamine and MK-801. J Pharmacol Exp Ther 250: 1019-1027

Koob GF (2004) A role for GABA mechanisms in the motivational effects of alcohol. Biochem Pharmacol 68: 1515-1525

Koob GF, Ahmed SH, Boutrel B, Chen SA, Kenny PJ, Markou A, O'Dell LE, Parsons LH, Sanna PP (2004) Neurobiological mechanisms in the transition from drug use to drug dependence. Neurosci Biobehav Rev 27: 739-749

Koob GF, Riley SJ, Smith SC, Robbins TW (1978) Effects of 6-hydroxydopamine lesions of the nucleus accumbens septi and olfactory tubercle on feeding, locomotor activity, and amphetamine anorexia in the rat. J Comp Physiol Psychol 92: 917-927

Korpi ER, Makela R, Romeo E, Guidotti A, Uusi-Oukari M, Furnari C, di Michele F, Sarviharju M, Xu M, Rosenberg PH (2001) Increased behavioral neurosteroid sensitivity in a rat line selectively bred for high alcohol sensitivity. Eur J Pharmacol 421: 31-38

Kralic JE, Wheeler M, Renzi K, Ferguson C, O'Buckley TK, Grobin AC, Morrow AL, Homanics GE (2003) Deletion of GABAA receptor alpha 1 subunit-containing receptors alters responses to ethanol and other anesthetics. J Pharmacol Exp Ther 305: 600-607

Krimmer EC, Schechter MD (1992) HAD and LAD rats respond differently to stimulating effect but not discriminative effects of ethanol. Alcohol 9: 71-74

Kuribara H (1994) Potentiation of the ambulation-increasing effect induced by combined administration of MK-801 with ethanol in mice. Psychopharmacology (Berl) 113: 453-456

Le Moal M, Simon H (1991) Mesocorticolimbic dopaminergic network: functional and regulatory roles. Physiol Rev 71: 155-234

Legault M, Wise RA (2001) Novelty-evoked elevations of nucleus accumbens dopamine: dependence on impulse flow from the ventral subiculum and glutamatergic neurotransmission in the ventral tegmental area. Eur J Neurosci 13: 819-828

Lewohl JM, Wilson WR, Mayfield RD, Brozowski SJ, Morrisett RA, Harris RA (1999) G-protein-coupled inwardly rectifying potassium channels are targets of alcohol action. Nat Neurosci 2: 1084-1090

Li TK, Lumeng L, McBride WJ, Murphy JM (1987) Rodent lines selected for factors affecting alcohol consumption. Alcohol Alcohol Suppl 1: 91-96

Liljequist S, Engel J (1982) Effects of GABAergic agonists and antagonists on various ethanol-induced behavioral changes. Psychopharmacology (Berl) 78: 71-75

Lin AM, Chai CY (1995) Dynamic analysis of ethanol effects on NMDA-evoked dopamine overflow in rat striatum. Brain Res 696: 15-20

Linakis JG, Cunningham CL (1979) Effects of concentration of ethanol injected intraperitoneally on taste aversion, body temperature, and activity. Psychopharmacology (Berl) 64: 61-65

Lister RG, File SE (1983) Performance impairment and increased anxiety resulting from the combination of alcohol and lorazepam. J Clin Psychopharmacol 3: 66-71

Lonnroth P, Jansson PA, Smith U (1987) A microdialysis method allowing characterization of intercellular water space in humans. Am J Physiol 253: E228-231

Lopez MF, Becker HC (2005) Effect of pattern and number of chronic ethanol exposures on subsequent voluntary ethanol intake in C57BL/6J mice. Psychopharmacology (Berl) 181: 688-696

Louis M, Clarke PB (1998) Effect of ventral tegmental 6-hydroxydopamine lesions on the locomotor stimulant action of nicotine in rats. Neuropharmacology 37: 1503-1513

Lovinger DM, White G (1991) Ethanol potentiation of 5-hydroxytryptamine3 receptor-mediated ion current in neuroblastoma cells and isolated adult mammalian neurons. Mol Pharmacol 40: 263-270

Lovinger DM, White G, Weight FF (1989) Ethanol inhibits NMDA-activated ion current in hippocampal neurons. Science 243: 1721-1724

Mague SD, Andersen SL, Carlezon WA, Jr. (2005) Early developmental exposure to methylphenidate reduces cocaine-induced potentiation of brain stimulation reward in rats. Biol Psychiatry 57: 120-125

Makanjuola RO, Ashcroft GW (1982) Behavioural effects of electrolytic and 6-hydroxydopamine lesions of the accumbens and caudate-putamen nuclei. Psychopharmacology (Berl) 76: 33-40

Martz A, Deitrich RA, Harris RA (1983) Behavioral evidence for the involvement of gamma-aminobutyric acid in the actions of ethanol. Eur J Pharmacol 89: 53-62

Mason ST, Corcoran ME, Fibiger HC (1979) Noradrenergic processes involved in the locomotor effects of ethanol. Eur J Pharmacol 54: 383-387

Masuzawa M, Nakao S, Miyamoto E, Yamada M, Murao K, Nishi K, Shingu K (2003) Pentobarbital inhibits ketamine-induced dopamine release in the rat nucleus accumbens: a microdialysis study. Anesth Analg 96: 148-152, table of contents

Mathe JM, Nomikos GG, Schilstrom B, Svensson TH (1998) Non-NMDA excitatory amino acid receptors in the ventral tegmental area mediate systemic dizocilpine (MK-801) induced hyperlocomotion and dopamine release in the nucleus accumbens. J Neurosci Res 51: 583-592

McClearn G, Rodgers D (1959) Differences in alcohol preference among inbred strains of mice. Quart. J. Stud. Alcoh. 20: 691-695

McGinnis JM, Foege WH (1999) Mortality and morbidity attributable to use of addictive substances in the United States. Proc Assoc Am Physicians 111: 109-118

McIntyre TD, Alpern HP (1985) Reinterpretation of the literature indicates differential sensitivities of long-sleep and short-sleep mice are not specific to alcohol. Psychopharmacology (Berl) 87: 379-389

McKay PF, Foster KL, Mason D, Cummings R, Garcia M, Williams LS, Grey C, McCane S, He X, Cook JM, June HL (2004) A high affinity ligand for GABAA-receptor containing alpha5 subunit antagonizes ethanol's neurobehavioral effects in Long-Evans rats. Psychopharmacology (Berl) 172: 455-462

McKee BL, Meshul CK (2005) Time-dependent changes in extracellular glutamate in the rat dorsolateral striatum following a single cocaine injection. Neuroscience 133: 605-613

McKinzie DL, Eha R, Cox R, Stewart RB, Dyr W, Murphy JM, McBride WJ, Lumeng L, Li TK (1998) Serotonin3 receptor antagonism of alcohol intake: effects of drinking conditions. Alcohol 15: 291-298

McMillan DE, Leander JD (1978) Food, water and ethanol consumption by rats under a fixed-interval schedule of food presentation. Drug Alcohol Depend 3: 227-234

McMillen BA, Williams HL (1998) Role of taste and calories in the selection of ethanol by C57BL/6NHsd and Hsd:ICR mice. Alcohol 15: 193-198

Meshul CK, Emre N, Nakamura CM, Allen C, Donohue MK, Buckman JF (1999) Time-dependent changes in striatal glutamate synapses following a 6-hydroxydopamine lesion. Neuroscience 88: 1-16

Messing RO, Carpenter CL, Diamond I, Greenberg DA (1986) Ethanol regulates calcium channels in clonal neural cells. Proc Natl Acad Sci U S A 83: 6213-6215

Meyer PJ, Palmer AA, McKinnon CS, Phillips TJ (2005) Behavioral sensitization to ethanol is modulated by environmental conditions, but is not associated with cross-sensitization to allopregnanolone or pentobarbital in DBA/2J mice. Neuroscience 131: 263-273

Meyer PJ, Phillips TJ (2003) Sensitivity to ketamine, alone or in combination with ethanol, is altered in mice selectively bred for sensitivity to ethanol's locomotor effects. Alcohol Clin Exp Res 27: 1701-1709

Minabe Y, Ashby CR, Jr., Schwartz JE, Wang RY (1991) The 5-HT3 receptor antagonists LY 277359 and granisetron potentiate the suppressant action of apomorphine on the basal firing rate of ventral tegmental dopamine cells. Eur J Pharmacol 209: 143-150

Mogenson GJ, M. BS, Wu M, Yang CR, Y. YCC (1993) From motivation to action: A review of dopaminergic regulation of limbic-nucleus accumbens-pedunculopontine nucleus circuitries involved in limbic-motor integration. In: Kalivas PW, Barnes CD (eds) Limbic Motor Circuits and Neuropsychiatry. CRC Press., Boca Raton, FL, pp 193-236

Mogenson GJ, Yang CR (1991) The contribution of basal forebrain to limbic-motor integration and the mediation of motivation to action. Adv Exp Med Biol 295: 267-290

Moghaddam B, Bolinao ML (1994) Biphasic effect of ethanol on extracellular accumulation of glutamate in the hippocampus and the nucleus accumbens. Neurosci Lett 178: 99-102

Molander A, Lof E, Stomberg R, Ericson M, Soderpalm B (2005) Involvement of accumbal glycine receptors in the regulation of voluntary ethanol intake in the rat. Alcohol Clin Exp Res 29: 38-45

Molander A, Soderpalm B (2005a) Accumbal strychnine-sensitive glycine receptors: an access point for ethanol to the brain reward system. Alcohol Clin Exp Res 29: 27-37

Molander A, Soderpalm B (2005b) Glycine receptors regulate dopamine release in the rat nucleus accumbens. Alcohol Clin Exp Res 29: 17-26

Nachman M, Lester D, Le Magnen J (1970) Alcohol aversion in the rat: behavioral assessment of noxious drug effects. Science 168: 1244-1246

Newland MC, Weiss B (1991) Ethanol's effects on tremor and positioning in squirrel monkeys. J Stud Alcohol 52: 492-499

Newlin DB, Thomson JB (1991) Chronic tolerance and sensitization to alcohol in sons of alcoholics. Alcohol Clin Exp Res 15: 399-405

Nie Z, Madamba SG, Siggins GR (1994) Ethanol inhibits glutamatergic neurotransmission in nucleus accumbens neurons by multiple mechanisms. J Pharmacol Exp Ther 271: 1566-1573

Nielsen DM, Crosley KJ, Keller RW, Jr., Glick SD, Carlson JN (1999) Left and right 6-hydroxydopamine lesions of the medial prefrontal cortex differentially affect voluntary ethanol consumption. Brain Res 823: 59-66

Nowak KL, McBride WJ, Lumeng L, Li TK, Murphy JM (1998) Blocking GABA(A) receptors in the anterior ventral tegmental area attenuates ethanol intake of the alcohol-preferring P rat. Psychopharmacology (Berl) 139: 108-116

O'Dell LE, Alomary AA, Vallee M, Koob GF, Fitzgerald RL, Purdy RH (2004) Ethanol-induced increases in neuroactive steroids in the rat brain and plasma are absent in adrenalectomized and gonadectomized rats. Eur J Pharmacol 484: 241-247

O'Donnell P, Greene J, Pabello N, Lewis BL, Grace AA (1999) Modulation of cell firing in the nucleus accumbens. Ann N Y Acad Sci 877: 157-175

Oades RD, Halliday GM (1987) Ventral tegmental (A10) system: neurobiology. 1. Anatomy and connectivity. Brain Res 434: 117-165

Oakley NR, Hayes AG, Sheehan MJ (1991) Effect of typical and atypical neuroleptics on the behavioural consequences of activation by muscimol of mesolimbic and nigro-striatal dopaminergic pathways in the rat. Psychopharmacology (Berl) 105: 204-208

Ohno T, Kanazawa I (1982) Mapping of the neural activity in the cerebellum of the mouse during stepping by means of 2-deoxyglucose autoradiography. Neurosci Lett 32: 119-123

Olds J, Milner P (1954) Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. J Comp Physiol Psychol 47: 419-427

Olive MF, Mehmert KK, Hodge CW (2000) Microdialysis in the mouse nucleus accumbens: a method for detection of monoamine and amino acid neurotransmitters with simultaneous assessment of locomotor activity. Brain Res Brain Res Protoc 5: 16-24

Olsen RW (1981) GABA-benzodiazepine-barbiturate receptor interactions. J Neurochem 37: 1-13

Paivarinta P, Korpi ER (1993) Voluntary ethanol drinking increases locomotor activity in alcohol-preferring AA rats. Pharmacol Biochem Behav 44: 127-132

Palmer AA, McKinnon CS, Bergstrom HC, Phillips TJ (2002a) Locomotor activity responses to ethanol, other alcohols, and GABA-A acting compounds in forward- and reverse-selected FAST and SLOW mouse lines. Behav Neurosci 116: 958-967

Palmer AA, Miller MN, McKinnon CS, Phillips TJ (2002b) Sensitivity to the locomotor stimulant effects of ethanol and allopregnanolone is influenced by common genes. Behav Neurosci 116: 126-137

Palmer AA, Moyer MR, Crabbe JC, Phillips TJ (2002c) Initial sensitivity, tolerance and cross-tolerance to allopregnanolone- and ethanol-induced hypothermia in selected mouse lines. Psychopharmacology (Berl) 162: 313-322

Papp M, Bal A (1987) Separation of the motivational and motor consequences of 6-hydroxydopamine lesions of the mesolimbic or nigrostriatal system in rats. Behav Brain Res 23: 221-229

Parker LA (1995) Rewarding drugs produce taste avoidance, but not taste aversion. Neurosci Biobehav Rev 19: 143-157

Pastor R, Miquel M, Aragon CMG (2005) Habituation to test procedure modeulates the involvement of dopamine D2- but not D1-receptors in ethanol-induced locomotor stimulation in mice. Psychopharmacology E-Publication

Paxinos, Watson (1997) The mouse brain in stereotaxic coordinates.

Pennartz CM, Groenewegen HJ, Lopes da Silva FH (1994) The nucleus accumbens as a complex of functionally distinct neuronal ensembles: an integration of behavioural, electrophysiological and anatomical data. Prog Neurobiol 42: 719-761

Pfeffer AO, Samson HH (1988) Haloperidol and apomorphine effects on ethanol reinforcement in free feeding rats. Pharmacol Biochem Behav 29: 343-350

Phillips PE, Robinson DL, Stuber GD, Carelli RM, Wightman RM (2003) Real-time measurements of phasic changes in extracellular dopamine concentration in freely moving rats by fast-scan cyclic voltammetry. Methods Mol Med 79: 443-464

Phillips TJ (1993) Use of genetically distinct mouse populations to explore ethanol reinforcement. Alcohol Alcohol Suppl 2: 451-455

Phillips TJ, Belknap JK, Buck KJ, Cunningham CL (1998) Genes on mouse chromosomes 2 and 9 determine variation in ethanol consumption. Mamm Genome 9: 936-941

Phillips TJ, Broadbent J, Burkhart-Kasch S, Henderson C, Wenger CD, McMullin C, McKinnon CS, Cunningham CL (2005) Genetic correlational analyses of ethanol reward and aversion phenotypes in short-term selected mouse lines bred for ethanol drinking or ethanol-induced conditioned taste aversion. Behav Neurosci 110

Phillips TJ, Burkhart-Kasch S, Gwiazdon CC, Crabbe JC (1992) Acute sensitivity of FAST and SLOW mice to the effects of abused drugs on locomotor activity. J Pharmacol Exp Ther 261: 525-533

Phillips TJ, Burkhart-Kasch S, Terdal ES, Crabbe JC (1991) Response to selection for ethanol-induced locomotor activation: genetic analyses and selection response characterization. Psychopharmacology (Berl) 103: 557-566

Phillips TJ, Crabbe JC, Metten P, Belknap JK (1994) Localization of genes affecting alcohol drinking in mice. Alcohol Clin Exp Res 18: 931-941

Phillips TJ, Huson M, Gwiazdon C, Burkhart-Kasch S, Shen EH (1995) Effects of acute and repeated ethanol exposures on the locomotor activity of BXD recombinant inbred mice. Alcohol Clin Exp Res 19: 269-278

Phillips TJ, Shen EH (1996) Neurochemical bases of locomotion and ethanol stimulant effects. Int Rev Neurobiol 39: 243-282

Phillips TJ, Shen EH, McKinnon CS, Burkhart-Kasch S, Lessov CN, Palmer AA (2002) Forward, relaxed, and reverse selection for reduced and enhanced sensitivity to ethanol's locomotor stimulant effects in mice. Alcohol Clin Exp Res 26: 593-602

Piepponen TP, Kiianmaa K, Ahtee L (2002) Effects of ethanol on the accumbal output of dopamine, GABA and glutamate in alcohol-tolerant and alcohol-nontolerant rats. Pharmacol Biochem Behav 74: 21-30

Pierce RC, Crawford CA, Nonneman AJ, Mattingly BA, Bardo MT (1990) Effect of forebrain dopamine depletion on novelty-induced place preference behavior in rats. Pharmacol Biochem Behav 36: 321-325

Pierce RC, Kumaresan V (2005) The mesolimbic dopamine system: The final common pathway for the reinforcing effect of drugs of abuse? Neurosci Biobehav Rev

Pierson J, Svenningsson P, Caprioli RM, Andren PE (2005) Increased levels of ubiquitin in the 6-OHDA-lesioned striatum of rats. J Proteome Res 4: 223-226

Pijnenburg AJ, van Rossum JM (1973) Letter: Stimulation of locomotor activity following injection of dopamine into the nucleus accumbens. J Pharm Pharmacol 25: 1003-1005

Plock N, Kloft C (2005) Microdialysis--theoretical background and recent implementation in applied life-sciences. Eur J Pharm Sci 25: 1-24

Pohorecky LA (1977) Biphasic action of ethanol. Biobehav Rev 1: 231-240

Ponomarev I, Crabbe JC (2002) A novel method to assess initial sensitivity and acute functional tolerance to hypnotic effects of ethanol. J Pharmacol Exp Ther 302: 257-263

Porrino LJ (1993) Functional consequences of acute cocaine treatment depend on route of administration. Psychopharmacology (Berl) 112: 343-351

Porrino LJ, Whitlow CT, Samson HH (1998) Effects of the self-administration of ethanol and ethanol/sucrose on rates of local cerebral glucose utilization in rats. Brain Res 791: 18-26

Purdy RH, Moore PH, Jr., Morrow AL, Paul SM (1992) Neurosteroids and GABAA receptor function. Adv Biochem Psychopharmacol 47: 87-92

Rajput AH, Jamieson H, Hirsh S, Quraishi A (1975) Relative efficacy of alcohol and propranolol in action tremor. Can J Neurol Sci 2: 31-35

Rasmussen K, Stockton ME, Czachura JF (1991) The 5-HT3 receptor antagonist zatosetron decreases the number of spontaneously active A10 dopamine neurons. Eur J Pharmacol 205: 113-116

Rebec GV, Christensen JR, Guerra C, Bardo MT (1997) Regional and temporal differences in real-time dopamine efflux in the nucleus accumbens during free-choice novelty. Brain Res 776: 61-67

Reid MS, Hsu K, Jr., Berger SP (1997) Cocaine and amphetamine preferentially stimulate glutamate release in the limbic system: studies on the involvement of dopamine. Synapse 27: 95-105

Rhodes JS, Best K, Belknap JK, Finn DA, Crabbe JC (2005) Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice. Physiol Behav 84: 53-63

Rice DP (1999) Economic costs of substance abuse, 1995. Proc Assoc Am Physicians 111: 119-125

Richter CP, Campbell KH (1940) Alcohol taste thresholds and concentrations of solution preferred by rats. Science 91: 507-509

Ripley TL, Jaworski J, Randall PK, Gonzales RA (1997) Repeated perfusion with elevated potassium in in vivo microdialysis--A method for detecting small changes in extracellular dopamine. J Neurosci Methods 78: 7-14

Risinger FO, Cunningham CL (1998) Ethanol-induced conditioned taste aversion in BXD recombinant inbred mice. Alcohol Clin Exp Res 22: 1234-1244

Risinger FO, Dickinson SD, Cunningham CL (1992) Haloperidol reduces ethanol-induced motor activity stimulation but not conditioned place preference. Psychopharmacology (Berl) 107: 453-456

Risinger FO, Malott DH, Prather LK, Niehus DR, Cunningham CL (1994) Motivational properties of ethanol in mice selectively bred for ethanol-induced locomotor differences. Psychopharmacology (Berl) 116: 207-216

Robbins TW, Everitt BJ (2002) Limbic-striatal memory systems and drug addiction. Neurobiol Learn Mem 78: 625-636

Robinson DL, Venton BJ, Heien ML, Wightman RM (2003) Detecting subsecond dopamine release with fast-scan cyclic voltammetry in vivo. Clin Chem 49: 1763-1773

Robinson DL, Volz TJ, Schenk JO, Wightman RM (2005) Acute ethanol decreases dopamine transporter velocity in rat striatum: in vivo and in vitro electrochemical measurements. Alcohol Clin Exp Res 29: 746-755

Rodd-Henricks ZA, McKinzie DL, Crile RS, Murphy JM, McBride WJ (2000) Regional heterogeneity for the intracranial self-administration of ethanol within the ventral tegmental area of female Wistar rats. Psychopharmacology (Berl) 149: 217-224

Roehrs TA, Samson HH (1981) Ethanol reinforced behavior assessed with a concurrent schedule. Pharmacol Biochem Behav 15: 539-544

Ross SB, Jackson DM, Wallis EM, Edwards SR (1988) Enhancement by a single dose of reserpine (plus alpha methyl-p-tyrosine) of the central stimulatory effects evoked by dopamine D-1 and D-2 agonists in the mouse. Naunyn Schmiedebergs Arch Pharmacol 337: 512-518

Rossetti ZL, Marcangione C, Wise RA (1998) Increase of extracellular glutamate and expression of Fos-like immunoreactivity in the ventral tegmental area in response to electrical stimulation of the prefrontal cortex. J Neurochem 70: 1503-1512

Rouge-Pont F, Mayo W, Marinelli M, Gingras M, Le Moal M, Piazza PV (2002) The neurosteroid allopregnanolone increases dopamine release and dopaminergic response to morphine in the rat nucleus accumbens. Eur J Neurosci 16: 169-173

Ryabinin AE, Criado JR, Henriksen SJ, Bloom FE, Wilson MC (1997) Differential sensitivity of c-Fos expression in hippocampus and other brain regions to moderate and low doses of alcohol. Mol Psychiatry 2: 32-43

Saigusa T, Tuinstra T, Koshikawa N, Cools AR (1999) High and low responders to novelty: effects of a catecholamine synthesis inhibitor on novelty-induced changes in behaviour and release of accumbal dopamine. Neuroscience 88: 1153-1163

Samson HH, Chappell A (2001) Muscimol injected into the medial prefrontal cortex of the rat alters ethanol self-administration. Physiol Behav 74: 581-587

Samson HH, Chappell A (2003) Dopaminergic involvement in medial prefrontal cortex and core of the nucleus accumbens in the regulation of ethanol self-administration: a dual-site microinjection study in the rat. Physiol Behav 79: 581-590

Samson HH, Czachowski CL (2003) Behavioral measures of alcohol self-administration and intake control: rodent models. Int Rev Neurobiol 54: 107-143

Sanchez FP, Dickenson L, George FR (1996) Ethanol self-administration is genetically independent of locomotor stimulation in fast and slow mice. Alcohol 13: 79-84

Sanchis-Segura C, Aragon CM (2002) Consequences of monosodium glutamate or goldthioglucose arcuate nucleus lesions on ethanol-induced locomotion. Drug Alcohol Depend 68: 189-194

Sanchis-Segura C, Correa M, Aragon CM (2000) Lession on the hypothalamic arcuate nucleus by estradiol valerate results in a blockade of ethanol-induced locomotion. Behav Brain Res 114: 57-63

Sanchis-Segura C, Pastor R, Aragon CM (2004) Opposite effects of acute versus chronic naltrexone administration on ethanol-induced locomotion. Behav Brain Res 153: 61-67

Sanders B, Sharpless SK (1978) Dissociation between the anticonvulsant action of alcohol and its depressant action in mice of different genotypes. Life Sci 23: 2593-2599

Sanders B, Sharpless SK, Collins AC, McClearn GE, Flanagan C (1978) Activating and anesthetic effects of general depressants. Psychopharmacology (Berl) 56: 185-189

Schaefer GJ, Holtzman SG (1979) Free-operant and auto-titration brain self-stimulation procedures in the rat: a comparison of drug effects. Pharmacol Biochem Behav 10: 127-135

Schaefer GJ, Michael RP (1987) Ethanol and current thresholds for brain self-stimulation in the lateral hypothalamus of the rat. Alcohol 4: 209-213

Schuckit MA (1980) Self-rating of alcohol intoxication by young men with and without family histories of alcoholism. J Stud Alcohol 41: 242-249

Schuckit MA (1985) Ethanol-induced changes in body sway in men at high alcoholism risk. Arch Gen Psychiatry 42: 375-379

Schuckit MA (1994) Low level of response to alcohol as a predictor of future alcoholism. Am J Psychiatry 151: 184-189

Schuckit MA, Gold EO, Croot K, Finn P, Polich J (1988) P300 latency after ethanol ingestion in sons of alcoholics and in controls. Biol Psychiatry 24: 310-315

Schuckit MA, Smith TL (2001) A comparison of correlates of DSM-IV alcohol abuse or dependence among more than 400 sons of alcoholics and controls. Alcohol Clin Exp Res 25: 1-8

Schuckit MA, Smith TL, Kalmijn J (2004) The search for genes contributing to the low level of response to alcohol: patterns of findings across studies. Alcohol Clin Exp Res 28: 1449-1458

Schultz W, Tremblay L, Hollerman JR (2003) Changes in behavior-related neuronal activity in the striatum during learning. Trends Neurosci 26: 321-328

Selim M, Bradberry CW (1996) Effect of ethanol on extracellular 5-HT and glutamate in the nucleus accumbens and prefrontal cortex: comparison between the Lewis and Fischer 344 rat strains. Brain Res 716: 157-164

Sellings LH, Clarke PB (2003) Segregation of amphetamine reward and locomotor stimulation between nucleus accumbens medial shell and core. J Neurosci 23: 6295-6303

Sesack SR, Pickel VM (1992) Prefrontal cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi and on dopamine neurons in the ventral tegmental area. J Comp Neurol 320: 145-160

Shellenberger MK, Gordon JH (1971) A rapid, simplified procedure for simultaneous assay of norepinephrine, dopamine, and 5-hydroxytryptamine from discrete brain areas. Anal Biochem 39: 356-372

Shen EH, Crabbe JC, Phillips TJ (1995) Dopamine antagonist effects on locomotor activity in naive and ethanol-treated FAST and SLOW selected lines of mice. Psychopharmacology (Berl) 118: 28-36

Shen EH, Dorow J, Harland R, Burkhart-Kasch S, Phillips TJ (1998) Seizure sensitivity and GABAergic modulation of ethanol sensitivity in selectively bred FAST and SLOW mouse lines. J Pharmacol Exp Ther 287: 606-615

Shen EH, Dorow JD, Huson M, Phillips TJ (1996) Correlated responses to selection in FAST and SLOW mice: effects of ethanol on ataxia, temperature, sedation, and withdrawal. Alcohol Clin Exp Res 20: 688-696

Shen EH, Phillips TJ (1998) MK-801 potentiates ethanol's effects on locomotor activity in mice. Pharmacol Biochem Behav 59: 135-143

Siegfried B, Filibeck U, Gozzo S, Castellano C (1982) Lack of morphine-induced hyperactivity in C57BL/6 mice following striatal kainic acid lesions. Behav Brain Res 4: 389-399

Silvestre JS, O'Neill MF, Fernandez AG, Palacios JM (1996) Effects of a range of dopamine receptor agonists and antagonists on ethanol intake in the rat. Eur J Pharmacol 318: 257-265

Sklar LS, Amit Z (1977) Manipulations of catecholamine systems block the conditioned taste aversion induced by self-administered drugs. Neuropharmacology 16: 649-655

Squires RF, Saederup E, Crawley JN, Skolnick P, Paul SM (1984) Convulsant potencies of tetrazoles are highly correlated with actions on GABA/benzodiazepine/picrotoxin receptor complexes in brain. Life Sci 35: 1439-1444

Stewart RB, Li TK (1997) The neurobiology of alcoholism in genetically selected rat models. Alcohol Health Res World 21: 169-176

Stewart RB, Perlanski E, Grupp LA (1988) Ethanol as a reinforcer for rats: factors of facilitation and constraint. Alcohol Clin Exp Res 12: 599-608

Stinchcomb A, Bowers BJ, Wehner JM (1989) The effects of ethanol and Ro 15-4513 on elevated plus-maze and rotarod performance in long-sleep and short-sleep mice. Alcohol 6: 369-376

Stratford TR, Kelley AE (1997) GABA in the nucleus accumbens shell participates in the central regulation of feeding behavior. J Neurosci 17: 4434-4440

Stricker ED, Zigmond MJ (1976) Recovery of function following damage to central catecholamine containing neurons; a neurochemical model of the lateral hypothalamic syndrome. In: J.M. S, A.N. E (eds) Progress in psychobiology and physiological psychology. Academic, New York, pp 121-189

Sugita R, Sawa Y, Nomura S, Zorn SH, Yamauchi T (1989) Effects of reserpine on dopamine metabolite in the nucleus accumbens and locomotor activity in freely moving rats. Neurochem Res 14: 267-270

Swanson LW (1982) The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Res Bull 9: 321-353

Swerdlow NR, Vaccarino FJ, Amalric M, Koob GF (1986) The neural substrates for the motor-activating properties of psychostimulants: a review of recent findings. Pharmacol Biochem Behav 25: 233-248

Tan AT, Dular R, Innes IR (1981) Alcohol feeding alters (3H)dopamine uptake into rat cortical and brain stem synaptosomes. Prog Biochem Pharmacol 18: 224-230

Tang A, George MA, Randall JA, Gonzales RA (2003) Ethanol increases extracellular dopamine concentration in the ventral striatum in C57BL/6 mice. Alcohol Clin Exp Res 27: 1083-1089

Teitelbaum H, Giammatteo P, Mickley GA (1979) Differential effects of localized lesions of n. accumbens on morphine- and amphetamine-induced locomotor hyperactivity in the C57BL/6J mouse. J Comp Physiol Psychol 93: 745-751

Tobler PN, Fiorillo CD, Schultz W (2005) Adaptive coding of reward value by dopamine neurons. Science 307: 1642-1645

Todtenkopf MS, Marcus JF, Portoghese PS, Carlezon WA, Jr. (2004) Effects of kappa-opioid receptor ligands on intracranial self-stimulation in rats. Psychopharmacology (Berl) 172: 463-470

Touchon JC, Moore C, Frederickson J, Meshul CK (2004) Lesion of subthalamic or motor thalamic nucleus in 6-hydroxydopamine-treated rats: effects on striatal glutamate and apomorphine-induced contralateral rotations. Synapse 51: 287-298

Tricklebank MD, Singh L, Oles RJ, Preston C, Iversen SD (1989) The behavioural effects of MK-801: a comparison with antagonists acting non-competitively and competitively at the NMDA receptor. Eur J Pharmacol 167: 127-135

Tzschentke TM, Schmidt WJ (2000) Functional relationship among medial prefrontal cortex, nucleus accumbens, and ventral tegmental area in locomotion and reward. Crit Rev Neurobiol 14: 131-142

Ueno S, Tsutsui M, Toyohira Y, Minami K, Yanagihara N (2004) Sites of positive allosteric modulation by neurosteroids on ionotropic gamma-aminobutyric acid receptor subunits. FEBS Lett 566: 213-217

Van Bockstaele EJ, Pickel VM (1995) GABA-containing neurons in the ventral tegmental area project to the nucleus accumbens in rat brain. Brain Res 682: 215-221

VanDoren MJ, Matthews DB, Janis GC, Grobin AC, Devaud LL, Morrow AL (2000) Neuroactive steroid 3alpha-hydroxy-5alpha-pregnan-20-one modulates electrophysiological and behavioral actions of ethanol. J Neurosci 20: 1982-1989

Ventura R, Alcaro A, Cabib S, Conversi D, Mandolesi L, Puglisi-Allegra S (2004) Dopamine in the medial prefrontal cortex controls genotype-dependent effects of amphetamine on mesoaccumbens dopamine release and locomotion. Neuropsychopharmacology 29: 72-80

Ventura R, Cabib S, Alcaro A, Orsini C, Puglisi-Allegra S (2003) Norepinephrine in the prefrontal cortex is critical for amphetamine-induced reward and mesoaccumbens dopamine release. J Neurosci 23: 1879-1885

Vezina P, Blanc G, Glowinski J, Tassin JP (1991) Opposed Behavioural Outputs of Increased Dopamine Transmission in Prefrontocortical and Subcortical Areas: A Role for the Cortical D-1 Dopamine Receptor. Eur J Neurosci 3: 1001-1007

Volkow ND, Wang GJ, Fischman MW, Foltin RW, Fowler JS, Abumrad NN, Vitkun S, Logan J, Gatley SJ, Pappas N, Hitzemann R, Shea CE (1997) Relationship between subjective effects of cocaine and dopamine transporter occupancy. Nature 386: 827-830

Volpicelli JR (2001) Alcohol abuse and alcoholism: an overview. J Clin Psychiatry 62 Suppl 20: 4-10

Wachtel H, Anden NE (1978) Motor activity of rats following intracerebral injections of drugs influencing GABA mechanisms. Naunyn Schmiedebergs Arch Pharmacol 302: 133-139

Wallace TL, Gudelsky GA, Vorhees CV (1999) Methamphetamine-induced neurotoxicity alters locomotor activity, stereotypic behavior, and stimulated dopamine release in the rat. J Neurosci 19: 9141-9148

Waller MB, Murphy JM, McBride WJ, Lumeng L, Li TK (1986) Effect of low dose ethanol on spontaneous motor activity in alcohol-preferring and -nonpreferring lines of rats. Pharmacol Biochem Behav 24: 617-623

Wang H, Pickel VM (2002) Dopamine D2 receptors are present in prefrontal cortical afferents and their targets in patches of the rat caudate-putamen nucleus. J Comp Neurol 442: 392-404

Wang MY, Rampil IJ, Kendig JJ (1999) Ethanol directly depresses AMPA and NMDA glutamate currents in spinal cord motor neurons independent of actions on GABAA or glycine receptors. J Pharmacol Exp Ther 290: 362-367

Weiss F, Porrino LJ (2002) Behavioral neurobiology of alcohol addiction: recent advances and challenges. J Neurosci 22: 3332-3337

Wise RA, Bozarth MA (1987) A psychomotor stimulant theory of addiction. Psychol Rev 94: 469-492

Wise RA, Hoffman DC (1992) Localization of drug reward mechanisms by intracranial injections. Synapse 10: 247-263

Wong LS, Eshel G, Dreher J, Ong J, Jackson DM (1991) Role of dopamine and GABA in the control of motor activity elicited from the rat nucleus accumbens. Pharmacol Biochem Behav 38: 829-835

Woodruff GN, Kelly PH, Elkhawad AO (1976) Effects of dopamine receptor stimulants on locomotor activity of rats with electrolytic or 6-hydroxydopamine-induced lesions of the nucleus accumbens. Psychopharmacologia 47: 195-198

Wright JM, Peoples RW, Weight FF (1996) Single-channel and whole-cell analysis of ethanol inhibition of NMDA-activated currents in cultured mouse cortical and hippocampal neurons. Brain Res 738: 249-256

Yan QS, Reith ME, Yan SG, Jobe PC (1998) Effect of systemic ethanol on basal and stimulated glutamate releases in the nucleus accumbens of freely moving Sprague-Dawley rats: a microdialysis study. Neurosci Lett 258: 29-32

Yim HJ, Gonzales RA (2000) Ethanol-induced increases in dopamine extracellular concentration in rat nucleus accumbens are accounted for by increased release and not uptake inhibition. Alcohol 22: 107-115

Yim HJ, Robinson DL, White ML, Jaworski JN, Randall PK, Lancaster FE, Gonzales RA (2000) Dissociation between the time course of ethanol and extracellular dopamine concentrations in the nucleus accumbens after a single intraperitoneal injection. Alcohol Clin Exp Res 24: 781-788

Yoshimoto K, Ueda S, Kato B, Takeuchi Y, Kawai Y, Noritake K, Yasuhara M (2000) Alcohol enhances characteristic releases of dopamine and serotonin in the central nucleus of the amygdala. Neurochem Int 37: 369-376

You ZB, Tzschentke TM, Brodin E, Wise RA (1998) Electrical stimulation of the prefrontal cortex increases cholecystokinin, glutamate, and dopamine release in the nucleus accumbens: an in vivo microdialysis study in freely moving rats. J Neurosci 18: 6492-6500

Yu D, Zhang L, Eisele JL, Bertrand D, Changeux JP, Weight FF (1996) Ethanol inhibition of nicotinic acetylcholine type alpha 7 receptors involves the amino-terminal domain of the receptor. Mol Pharmacol 50: 1010-1016

Yun IA, Wakabayashi KT, Fields HL, Nicola SM (2004) The ventral tegmental area is required for the behavioral and nucleus accumbens neuronal firing responses to incentive cues. J Neurosci 24: 2923-2933

Zacny JP, Lichtor JL, Flemming D, Coalson DW, Thompson WK (1994) A dose-response analysis of the subjective, psychomotor and physiological effects of intravenous morphine in healthy volunteers. J Pharmacol Exp Ther 268: 1-9

Zetterstrom T, Fillenz M (1990) Local administration of flurazepam has different effects on dopamine release in striatum and nucleus accumbens: a microdialysis study. Neuropharmacology 29: 129-134

Zocchi A, Girlanda E, Varnier G, Sartori I, Zanetti L, Wildish GA, Lennon M, Mugnaini M, Heidbreder CA (2003) Dopamine responsiveness to drugs of abuse: A shell-core investigation in the nucleus accumbens of the mouse. Synapse 50: 293-302