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How Addictive Drugs Disrupt Presynaptic Dopamine Neurotransmission

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Abstract

The fundamental principle that unites addictive drugs appears to be that each enhances synaptic dopamine by means that dissociate it from normal behavioral control, so that they act to reinforce their own acquisition. This occurs via the modulation of synaptic mechanisms involved in learning, including enhanced excitation or disinhibition of dopamine neuron activity, blockade of dopamine reuptake, and altering the state of the presynaptic terminal to enhance evoked over basal transmission. Amphetamines offer an exception to such modulation in that they combine multiple effects to produce non-exocytic stimulation-independent release of neurotransmitter via reverse transport independent from normal presynaptic function. Questions on the molecular actions of addictive drugs, prominently including the actions of alcohol and solvents, remain unresolved, but their ability to co-opt normal presynaptic functions helps to explain why treatment for addiction has been challenging.

1. INTRODUCTION

Addiction is an unusual disease in that it is not a consequence of cellular dysfunction: addictive drugs “hijack” normal learning processes to reinforce their own acquisition. It is further unusual in being a modern disorder: Roy Wise made the observation in this journal that “Addiction is quite a recent phenomenon, largely dependent upon the controlled use of fire (smoking), hypodermic syringes (intravenous injection), and the cork and bottle (storage and transportation of alcohol)” (Wise, 2000). To more efficient delivery systems, we add the contributions of modern chemists, who isolated active components of psychoactive plants (cocaine and morphine) and invented easily administered drugs (amphetamine: AMPH, methamphetamine: METH, toluene, and heroin).

Addictive drugs exhibit a wide range of structures and actions, but the unifying principle appears to be that they each acutely enhance striatal dopamine (DA) neurotransmission by means that dissociate it from normal drive by environmental cues.

Striatal DA levels are normally driven by three major factors: 1) *neuronal firing*, which is chiefly modulated by environmental cues via somatodendritic receptors; 2) *reuptake* by the DA plasma membrane uptake transporter (DAT); 3) the *state of the presynaptic terminal*,

which controls the number, probability, and size of the quantal events released in response to neuronal activity.

Thus, addictive drugs might in principle act by 1) *enhancing neuronal firing* beyond that normally driven by environmental cues, as do nicotine, opiates, and sedatives: 2) by *inhibiting DA reuptake*, as does cocaine; 3) *altering release probability* from the presynaptic terminal, as do nicotine and opiates. Less predictable from normal synaptic function are the actions of AMPHs, which 4) *release DA via reversal of DAT* independent of synaptic vesicle fusion (Table 1).

The concept and identification of addictive drugs

Defining the set of addictive drugs is elusive in part as it depends on whether society considers them to be destructive. For example, evidence for clinical opium use extends to 5000 BC in Sumeria, where it was known as “joy plant”; among a horde of opium-related artifacts surviving from the ancient Mediterranean is a goddess from Minoan Crete with a poppy crown associated with a vase apparently used for breathing opium vapors (Kritkikos and Papdaki, 1967). Ancient and medieval Greek and Arabic pharmacologists who wrote on opium including Dioscorides, Galen, and Avicenna (Ibn Sina) mention opium’s clinical use and toxicity but not habit (Tibi, 2006).

Recreational opium smoking was established in China in the 1600s (Tibi, 2006), while opium was mostly eaten in Turkey. The first western scientific article on opium that I have located is in 1701 by Dr. John Jones, from the London College of Physicians (Jones, 1701) who discusses clinical uses, as well as withdrawal symptoms, including death, after “lavish use”.

In the second western medical article on opium (Awsiter, 1763), Dr. John Awsiter of the Royal Hospital in Greenwich introduced the term *habit*, stating “there are many properties in it, if universally known, that would habituate the use, and make it more in request with us that the Turks themselves, the result of which knowledge must prove a general misfortune.” He compared opium’s effects to drunkenness and outlines features of *withdrawal* and *overdose*. He further discussed *tolerance* of large doses by those with opium habit. He also wrote of *dire consequences for society*: “the lives of the major part of the Eastern countries, where it is so much requested, would drop in the flower of their youth, and whole nations in the space of a century, be depopulated.” He further presaged treating one habit forming drug with others by prescribing nervous stimulants, which would have included ethanol or camphor (Sneader, 1990). It may be that the contemporary concept of addiction begins here.

The concept of uncontrollable drug habit was popularized by satirist Thomas De Quincey in “Confessions of an English Opium Eater” (1822). The notion of addiction as a mass social scourge was reinforced in reaction to the British government’s efforts to promulgate the opium trade, forcing farmers in India to grow the crop and initiating two wars with China to enforce its ability to export it (1839–1842 and 1856–1860). These actions promulgated a protest movement in Britain (Howitt, 1839) that included political and medical arguments: a medical tract by the “Society for the Suppression of the Opium Trade” (Bennett, 1882) includes articles by doctors who together treated thousands of opium addicts in China and stresses the dire consequences for society.

Addiction labeled as a disease was further associated with the adoption of the hypodermic syringe during the American Civil War, which was said to engender a “taste” for morphine and opium, as well as the growing temperance movement against alcohol. To encourage scientific study of addiction, American medical doctors formed the American Association for the Study and Cure of Inebriety (AASCI), which published the *Journal of Inebriety* from

1876 to 1914. An article in tthe January 8, 1886 issue of *Science* on work by Asa Meylert, attributed “many deaths of patients in hospitals and asylums, and of soldiers on the march, to the sudden deprivation of opium to which they have been accustomed”. Meylert asked for addiction to be treated as “a disease, which must be treated as other diseases are, by appropriate remedies.” The muscarinic antagonist atropine was already being used for treatment of opium addiction, but Meylert reported that while it and coca were ineffective, marijuana, the glycine receptor antagonist strychnine, the muscarinic antagonist henbane, quinine, and inhalant anesthetic chloroform were indicated.

Cocaine which followed morphine (Sertuerner, 1817) as an addictive drug introduced by modern chemists, is the active agent of the coca leaf (Gaedcke, 1855), which has been cultivated for thousands of years and is not considered to be addictive. By 1863, cocaine was being sold to the public, including in Coca-Cola in 1886: this beverage still continues to contain coca leaf (Time Magazine, May 25, 2009).

The still running argument over cocaine’s addictive qualities was covered in the April 8, 1887 issue of *Science* in a discussion between Brooklyn physicians Dr. J.B. Mattison and Dr. Hammond. Mattison presented a long list or patients with cocaine toxicity, and insisted that “Hammond’s assertion that there is no danger of cocaine addiction because he himself took half a dozen doses at intervals of from one to four days” insufficient evidence against addiction. The June 4, 1887 issue of the *British Medical Journal* introduced the term drug “craze” to the scientific literature in an article on Mattison’s presentation.

In summary, the idea of addiction as disease seems to have coalesced during the century after Awsiter’s article on opium. The opiates, cocaine, and ethanol each fulfill Aswiter’s criteria, as may some AMPHs, some solvents including toluene and ether, barbituates and arguably benzodiazepines. Most individuals who have taken these drugs, however, do not become addicted. A less classic example is tobacco, which is thought to not exhibit induce tolerance to its rewarding effects. Additional drugs that may fall into this category include phencyclidine, betel nut, marijuana, caffeine, γ-hydroxybutyrate, and “hallucinogens” including yage, psilocybin, and LSD: these are not so widely considered to be addictive, but this may change. We do not review metabolites and combinatorial properties of addictive drugs, although these are quite interesting: for instance chloral hydrate, a component of the Mickey Finn, is metabolized to the active ethanol metabolite tricloroethanol, while cocaine and ethanol can react to produce cocaethylene, which may be more reinforcing than either individual component.

Identification of a role for DA neurotransmission in addiction

Identification of a “reward pathway”—The classic behaviorist B.F. Skinner avoided the term “reward”, and there are good reasons for this. Here I use it as the neuroscience literature does, which is similar or identical to positive reinforcement, and not to imply that DA neurotransmission is a cause of pleasure. I do not distinguish the substantia nigra (SN) from ventral tegmental area (VTA) ventral midbrain DA neurons or the dorsal from the ventral striatum/nucleus accumbens (nAc) unless important to the discussion: a recent review discusses current controversy on the roles of these groups (Wise, 2009).

Credit for discovering that striatal DA neurotransmission is fundamental to drug self-administration is based on the confluence of two independent groups using different means: James Olds and colleagues in the 1950’ and 60’s, and the pharmacologists associated with Aarvid Carlsson including Nils Hillarp, Annica Dahlstrom, Kjell Fuxe, and Urban Ungerstedt who were establishing DA as a neurotransmitter.

In 1954, Olds and Peter Milner introduced intracranial self-stimulation by implanting electrodes in the brains of rats and providing them with a lever that they could press to apply current (Olds and Milner, 1954). Soon after, Olds introduced “intracranial self-administration”, a lever-operated device that would allow rats to inject drugs via a pipette directly into defined areas of the brain (Olds and Olds, 1958). The self-stimulation paradigm provided the initial evidence of how activity in specific brain regions correlated with the fraction of the time the animals pressed the lever, while self-administration provided insights into drug effects at specific brain regions.

Initial experiments demonstrated that rats with electrodes in the septal area used as much as 92% of the time to bar press, in contrast to regions where “animals do everything possible to avoid stimulation.” By 1956, Olds and collaborators found that stimulation of the hypothalamus was even more rewarding, eliciting as many as 5000 bar presses per hour. They soon suspected that the efficacy of stimulation of the lateral hypothalamus was due to activation of the medial forebrain bundle, through which DA neurons course from cell bodies in the midbrain to striatal and cortical targets.

They then attempted to interfere with bar presses by administering drugs (Olds et al., 1956). Successful inhibitors of self-administration included reserpine, which block uptakes of catecholamines into synaptic vesicles (Carlsson et al., 1962; Kirshner, 1962) and chlorpromazine, the antipsychotic, which Carlsson later showed to block DA receptors.

By 1958, Olds concluded: “(i) The cells which mediate primary rewarding effects are located in a midline system running from the midbrain through and into the subcortical and cortical groups of the rhinencephalon. (ii) The cell groups which mediate primary rewarding effects are different from those which mediate primary punishing effects” (Olds, 1958). They validated the role of medial forebrain projection from the midbrain to the cortical and subcortical areas in these rewarding effects in later lesion studies (Olds and Olds, 1969).

Also in 1969, Olds and Phillips introduced the concept that salient stimuli are responsible for the firing of DA neurons by showing that ventral midbrain neurons fired at a higher frequency following a tone paired with food presentation, and lower rates following other tones not paired with a reinforcer (Phillips and Olds, 1969). Remarkably, they conclude with essentially the contemporary understanding of the rules of DA neuronal firing: “Thus it was surmised that expectancy of reward, rather than response to the tones per se, accounted for the differing rates of firing in midbrain unit activity … These responses reflect an integration of sensory input with the internal state, where the response to tones which signified a reward appropriate to the submotivational state of the organism was amplified by the degree of that motivation.“ These insights, along with a posthumous study that outlines a specific role for VTA neurons (Brauth and Olds, 1977), promulgated subsequent explorations to decipher the rules by which reinforcement control the activity of DA neurons (*cite Schultz review this issue*).

By 1976, the year that he died in an accident, Olds wrote a review on the state of self-stimulation and drive, concluding that “noradrenaline neurons might be the reward neurons addressed to negative drives and DA neurons to positive drives” (Olds, 1976). By introducing self-stimulation paradigms using electrical current and direct drug application, exploiting this to map the brain regions involved, and characterizing firing modes by these neurons to reward and stimuli associated with reward, Olds perhaps provided the strongest contribution to our understanding of addiction.

Evidence that addictive drugs enhance striatal DA neurotransmission—During this same period, Carlsson’s colleagues developed histochemical fluorescent techniques

showing that DA neurons originated in the ventral mesencephalon and projected to the cortex and striatum (Hillarp et al., 1966), in the pathway Olds had identified. Similarities between the behavioral response to AMPH and electric self-stimulation of this pathway were observed, and led Crow and colleagues to suggest that "the dopamine-containing system arising from the ventral mesencephalon may function as an activating system involved in the effects of positive reward on operant behaviour" (Anlezark et al., 1971). A variety of neurochemical experiments to measure catecholamine release during self-stimulation were conducted, as well as additional lesioning studies, as reviewed (German and Bowden, 1974).

Experiments by Wise, Fibiger, Phillips and others were influential in convincing the field that DA release was particularly important for reward, for example by showing that partial DA receptor blockade increased self administration of AMPH by rats, while rats would self administer direct DA receptor agonists (Yokel and Wise, 1978). The memorably named De Wit and Wise (1977) showed that a DA D2 receptor antagonist, but not norepinephrine antagonists, blocked cocaine reinforcement.

An approach that convinced the field at large that DA was responsible for the actions of addictive drugs was in vivo microdialysis using electrochemical detection. DA release in vivo had been studied using "push-pull cannula", but this caused significant tissue damage, while voltammetry (Kissinger et al., 1973) measured catecholamine release and reuptake with extraordinary time resolution (Millar et al., 1985; Rice et al., 1985) and was effective for studying cocaine and AMPH (Caviness and Wightman, 1982; Ewing et al., 1983), but could not differentiate between electroactive compounds (Gonon et al., 1980) including norepinephrine and DA. Microdialysis with HPLC electrochemical detection was pioneered by Ralph Adams and collaborators (Adams, 1976; Plotsky et al., 1977), who showed that AMPH released DA, and Ungerstedt's lab (Ungerstedt and Pycock, 1974), who confirmed this response in the striatum.

Microdialysis studies were extended by Assunta Imperato and Gaetano di Chiara and colleagues, who demonstrated that ethanol (Di Chiara and Imperato, 1985), opiates and barbituates (Di Chiara and Imperato, 1986), and nicotine (Imperato et al., 1986) increased DA concentrations in striatum, particularly in the ventral striatum/nAc. Drugs with aversive properties decreased DA release, and non-abused drugs did not modify synaptic DA (Di Chiara and Imperato, 1988a).

Most recently, studies in human stimulant-naïve individuals have shown that AMPH-mediated DA release as measured by D2 receptor availability is significantly associated with effects of the drug reported by the subject as "happiness" and "energy" (Abi-Dargham et al., 2003).

In summary, a role for enhanced striatal DA neurotransmission in the addictive properties of drugs is supported by:

1. An increase in extracellular DA levels in the striatum measured following all of the classic addictive drugs
2. An increased level of drug self-administration when DA receptors are partially antagonized, with cessation at more complete blockade
3. Inhibition of drug self-administration when catecholamines release is decreased by VMAT inhibition or when DA synthesis is blocked
4. Cessation of self-administration when DA neurons or their axons are ablated

5. Reports by AMPH naïve-individuals providing a correlation between DA release and self-reported euphoria

Further analysis reveals that each mechanism of action essentially decouples DA release from normal physiological control. From Olds' studies, this would be expected to provide positive stimuli associated with acquiring the drug, providing a conceptual synaptic framework for addiction.

Physiological regulation of striatal extracellular DA

Multiple current reviews detail the control of extracellular DA levels in the striatum (Arbuthnott and Wickens, 2007; Rice and Cragg, 2008; Sulzer et al., 2010) and new electrochemical approaches in vivo are more precisely measuring the effects of midbrain DA neuron firing on striatal DA levels (Sombers et al., 2009).

Succinctly, midbrain DA neurons fire action potentials in two patterns. The intrinsic *tonic* firing pattern exhibits a mean frequency of ~ 4 Hz in the rodent (Grace and Bunney, 1984; Sanghera et al., 1984) and is maintained in VTA neurons by HCN channels (Chan et al., 2007) and an after-hyperpolarization from Ca^{2+}-activated apamin-sensitive K+ channels that delay a return to threshold (Shepard and Bunney, 1991; Lovejoy et al., 2001). Superimposed on this activity in vivo are *phasic bursts* of two to six action potentials at a frequency of ~ 15 Hz in rodents. The burst firing is due to the confluence of glutamatergic inputs including superior colliculus (Coizet et al., 2006), the pedunculopointine tegmental nucleus (PPT) (Lokwan et al., 2000), lateral dorsal tegmental nucleus (Lodge and Grace, 2006), subthalamic nucleus (Iribe et al., 1999; Chatha et al., 2000), the prefrontal cortex (Tong et al., 1996), and additional areas (Geisler et al., 2007), and is absent in acute midbrain slices. Enhanced firing is triggered by conditioned stimuli (Phillips and Olds, 1969) and sensory (Freeman and Bunney, 1987) stimuli in rats, and by appetitive or conditioned stimuli in monkey (Mirenowicz and Schultz, 1996). Downstream from the initial effects on addictive drugs on dopamine neurotransmission discussed in this review, relatively long term, presumably DA-dependent, alterations of plasticity at these excitatory inputs, such as long term potentiation, are suspected to play a role in establishing addiction-related behaviors (*refer to Malenka & Luscher review in this issue*). As detailed below, nicotine's actions are in part due to activation of these synaptic inputs.

Burst firing of VTA DA neurons is depressed by activity of inhibitory GABAergic inputs, including striatonigral projections to the SN reticulata that interact with DAergic dendrites of the SN, the ventral pallidum, the collaterals of ventral midbrain projection neurons and a major input from mesopontine rostromedial tegmental nucleus (Jhou et al., 2009; Kaufling et al., 2009; Sesack and Grace, 2010). As detailed below, effects on these inhibitory synapses play important roles in the actions of opioids, benzodiazepines, and nicotine.

Modulatory inputs to DA neurons in the ventral midbrain include cholinergic neurons arriving from the PPT and lateral dorsal tegmental nucleus, which make synapses onto both DA and GABAergic neurons (Omelchenko and Sesack, 2006), as well as serotonin, norepinephrine, orexin, and other peptide transmitters.

These firing patterns interact with the probability of release from DA terminals and DAT activity to regulate extracellular levels. The baseline or "tonic" DA level in the striatum appears to mostly reflect equilibria due to combined tonic activity and DAT action. Current estimates of the baseline level in the striatum are ~20 nM (Shou et al., 2006).

The rapid response of carbon fibre electrodes allows measurement of DA release following a single electrical stimulation. A single pulse electrical stimulation of the medial forebrain bundle (MFB) elicits ~ 400 nM DA in anesthetized mouse striatum (Gonon, 1997). The

burst firing of the midbrain DA neurons cause sufficient DA release over tonic firing so that DAT is saturated during the bursts. This increases the duration of the enhanced striatal DA levels and provides far greater levels of DA per action potential during bursts than during tonic firing.

The level of DA reached in awake animals, where burst firing or self-stimulation occurs, appears similar. The average maximum of spontaneous transients in freely moving rats is ~50 nM DA (Wightman et al., 2007). Intracranial self-stimulation in rats can elicit DA levels in the nAc > 2 μM (Garris et al., 1999), while behavioral stimuli elicit 200–500 nM (Robinson et al., 2001). Due to the dead volume around the electrode, these measurements likely underestimate maximum levels by 2–3 fold (Sulzer and Pothos, 2000).

Important additional local control in the striatum is determined by release probability at the DA terminals, which is controlled by nAChR (see nicotine discussion), muscarinic nicotine receptors (Bendor et al., 2010), GABA receptors (Schmitz et al., 2002), D2 DA receptors (Benoit-Marand et al., 2001; Phillips et al., 2002; Schmitz et al., 2002), and likely additional presynaptic receptors, some regulated by complex network properties (Bamford et al., 2008), which principally act by modulation presynaptic Ca^{2+}.

Amphetamines

AMPH and its many derivatives might be considered the orchids of psychoactive drugs. In addition to the herbs khat and ephedra, the active components of which (cathinone and ephedrine) have an AMPH-like structure, nearly 200 substituted AMPHs have been introduced (Nichols, 1994) including METH, methylphenidate (Ritalin), and MDMA (ecstacy).

AMPHs encompass the only widely administered class of drugs that predominantly release neurotransmitter by a non-exocytic mechanism. Multiple effects of AMPH act together to provide this unique mechanism of action, and these complexities provide the reason that its discussion is the longest here: for more detail refer to a recent review (Sulzer et al., 2005).

AMPH, invented in 1887 (Edeleano, 1887), was bequeathed its generic name from a contraction of alpha-methyl-phenethyl-amine. Some notion of its colorful history can be seen from the 1989 Merck Index listing, which lists seventeen trade names, not including such familiar trade names as Adderall, Benzedrine, and Dexedrine, or its myriad nicknames. AMPH was introduced commercially in 1932 as Benzedrine, the free base administered in inhaler form. In 1936, Smith Kline and French began to sell Benzedrine without prescription: over 50 million tablets were sold during the first three years of availability. It is said that the first widespread use of AMPH spread from campus to campus following experiments by the Department of Psychology at the University of Minnesota on alertness in college students (Angrist and Sudilovsky, 1978). AMPH was made available by prescription only on January 1, 1939. AMPH, METH, and methylphenidate are still widely prescribed for weight control, narcolepsy, and attention deficit disorder, with 700,000 Americans currently taking Adderall, a mixture of S(+) and R(−)AMPH enantiomers (Forrester, 2007).

The addictive potential of AMPH was mentioned in 1937 (Guttmann and Sargeant, 1937) but was a topic of debate (Angrist and Sudilovsky, 1978) and not fully recognized until the mid-1960's (Lemere, 1966). AMPH abuse tends to occur in epidemic waves, as in Japan from 1947–1957, when 550,000 Japanese were using the drug illicitly (Fukui et al., 1994). In Sweden in 1942, four years following its introduction, an estimated 3% of the country's population used AMPH (Rylander, 1972). In the 2007 U.S. National Survey on Drug Use and Health, the fraction of people in Western States that take METH was 10-fold higher than in the Northeast. Epidemics are reported for gay and bisexual men (Urbina and Jones,

2004), and it is associated with a high incidence of AIDS in HIV+ patients, perhaps in part as its weak base properties inhibit normal antigen presentation (Talloczy et al., 2008).

The amount of METH or AMPH in the brain probably reaches 5–10 μM following clinical administration (Markov et al., 2008) but may reach the hundreds of μM following self-administration in tolerant addicts, who can ingest 3–4 grams over a 6 day period (Talloczy et al., 2008), which may engender greater toxic response. AMPH and METH showed no differences in terms of changes in DA release in the dorsal striatum, elimination, or other pharmacokinetic properties (Melega et al., 1995), and the drugs are not distinguished in human discrimination studies (Lamb and Henningfield, 1994). While the occasional statement that METH is more addictive or potent may be unfounded (Shoblock et al., 2003), METH was reported to be more effective at increasing DA levels in the nAc but not dorsal striatum in rats (Goodwin et al., 2009).

AMPH effects both uptake transporters and secretory vesicles

Inquiry into pharmacological stimulant mechanisms was initiated by George Barger and Henry H. Dale (Barger and Dale, 1910) who found that compounds including β-phenylethylamine, and the AMPH isomers, beta-methylphenethylamine and phenylpropylamine, raise blood pressure. They called such compounds *sympathomimetic*. In later work by J.H. Burn and colleagues, sympathomimetics that caused membrane contraction after sympathetic postganglionic denervation and were not blocked by reserpine were labeled *directly acting*, in contrast to *indirectly acting* sympathomimetics that required innervation to produce contraction and had reserpine-sensitive responses (Burn and Rand, 1958; Fleckenstein and Burn, 1953). They concluded that tyramine and AMPH, which were in the second class, “act in the normal animal by releasing a noradrenaline-like substance”, the first clear declaration that AMPHs act by releasing catecholamines.

From the mid-1960’s through the 70’s, a variety of studies showed that AMPH also released catecholamines in the CNS, including DA (Heikkila et al., 1975), as reviewed (Kuczenski and Segal, 1994). Ritz and Kuhar pointed out that while self-administration of cocaine-like blockers correlated with their binding efficacy to DAT, AMPH was far more potent than would be expected from its binding, confirming the findings in the periphery that release rather than reuptake blockade is most important for AMPH action (Ritz et al., 1987).

A role for synaptic vesicle DA pools was sometimes doubted due to contradictory results from reserpine experiments. This issue was re-addressed using transformed cells to express DAT, the neuronal vesicular monoamine transporter (VMAT2), or both (Pifl et al., 1995). AMPH released DA only in cells that expressed DAT, but the release was greater and sustained for cells that coexpressed VMAT, confirming a role for synaptic vesicle DA stores. Consistently, cyclic voltammetry studies demonstrated that knockout mice that do not express DAT did not exhibit AMPH-mediated DA release (Giros et al., 1996; Jones et al., 1998), while AMPH-mediated DA release from cultured VMAT knockout ventral midbrain neurons was depressed by 65% (Fon et al., 1997). As entire neurotransmitter pool can be measured in the cultures, AMPH was moreover found to rapidly (30 min) increase DA synthesis. Along with the drug’s inhibition of MAO, this provided most of the source for the remaining DA release from VMAT2 knockout neurons (Larsen et al., 2002). Thus, there is evidence for cytosolic, vesicular, and newly synthesized pools that contribute to the DA released by AMPH,

Perhaps the strongest evidence that synaptic vesicle and cytosolic DA pools both contribute to AMPH action was provided by cyclic voltammetry studies in striatal brain slice by Sara Jones and colleagues (Jones et al., 1998). They stimulated the terminals at regular intervals and perfused AMPH, which provided a rapid, relatively small amount of baseline, i.e.,

unstimulated, DA release. Minutes later, the amount of DA released per electrical pulse decreased while the baseline release became much larger. Thus, it appeared that most released DA was initially in synaptic vesicles and had been redistributed to the cytosol for subsequent reverse transport. A later study showed that a portion of the decrease in evoked DA release was due to an inhibitory D2 DA receptor feedback mechanism (Schmitz et al., 2001), but was still consistent with a role for DA that previously resided in synaptic vesicles.

Some reports suggested the notion that low concentrations of AMPH preferentially release catecholamine already resident in the cytosol, whereas higher concentrations are required to redistribute vesicular catecholamine to the cytosol (Seiden et al., 1993). If AMPH simply releases cytosolic catecholamine, then the free catecholamine levels in the cytosol should decrease. On the other hand, if AMPH redistributes catecholamine from vesicles to the cytosol, the free cytosolic levels might increase. Testing these predictions has required a means to measure cytosolic catecholamines. An initial approach measured free cytosolic DA in a giant DA neuron in the pond snail *Planorbis corneus* (Sulzer et al., 1995); exposure to 10 μM AMPH increased cytosolic DA, consistent with redistribution of vesicular DA to the cytosol. When AMPH accumulation by plasma membrane transporters was skirted by an intracellular injection of ~100 μM AMPH, there was an increase in cytosolic DA within 5 sec, indicating that the effects on vesicles are quite rapid.

More recently, Mosharov and colleagues developed intracellular patch electrochemistry to measure cytosolic catecholamine levels in chromaffin cells (Mosharov et al., 2003) and neurons (Mosharov et al., 2009). In chromaffin cells, they found that 10 μM AMPH induced a 15-fold increase in cytosolic DA within 10–15 min of exposure, indicating a redistribution of vesicular catecholamines. In contrast, in cell bodies of cultured ventral midbrain DA neurons, METH decreased cytosolic DA unless cocaine was added, which apparently blocked reverse transport (Mosharov et al., 2009). Thus, in chromaffin cell bodies, AMPH redistributes vesicular DA to the cytosol, but this does not occur in DA neuronal cell bodies as they lack synaptic vesicles. There presumably is such redistribution from vesicles to cytosol in presynaptic terminals, but these are too small to be patched, and so optical approaches are now being pursued.

AMPH actions on synaptic vesicles

AMPH and METH have long been known to displace catecholamines, but not ATP, from suspended chromaffin vesicles (Carlsson et al., 1963; Schumann and Philippu, 1962). Uptake of norepinephrine into isolated small synaptic vesicles was later shown to be inhibited by AMPH and AMPH analogs (Knepper et al., 1988).

If AMPH redistributes DA from synaptic vesicles, it should decrease the amount of transmitter released per secretory vesicle fusion event, i.e., “the quantal size.” This became testable with the development of carbon fiber electrodes capable of recording quantal exocytosis by amperometry (Wightman et al., 1991). Using this technology, AMPH provided the first instance of a manipulation that affected the quantal size of catecholamine release, as amperometric recordings in PC12 cells, an adrenal chromaffin cell-derived cell line, demonstrated that 10 μM of AMPH for 10 min decreased quantal size by 50% (Sulzer et al., 1995). Decreased quantal size by AMPH and other weak bases was later confirmed in chromaffin cells (Mundorf et al., 1999) and the giant DA neuron of freshwater snail where Ewing and colleagues demonstrated the existence of two classes of DA vesicles differentially depleted by AMPH (Anderson et al., 1998).

There are at least two non-exclusive hypotheses that may explain the mechanism by which AMPH redistributes vesicular monoamines to the cytosol, the *weak base hypothesis* and *VMAT substrate actions*.

The weak base hypothesis—All sympathomimetics are weak bases with amine moieties that are capable of accepting protons with pKs in the range of ~8 to 10 (Sulzer and Rayport, 1990): AMPH is a lipophilic weak base with a pK of 9.9 and is thus protonated in acidic organelles including catecholamine vesicles (Sulzer and Rayport, 1990): once charged, it is less membrane permeable and accumulates in the acidic structure.

The acidic pH gradient in secretory vesicles provides the energy to accumulate transmitter against its concentration gradient (Johnson, 1988). Secretory vesicles are acidic; chromaffin vesicles, which are the best characterized, maintain a pH of 5.0 – 5.7, depending on conditions (Johnson, 1988; Lee et al., 2010; Markov et al., 2008; Pothos et al., 2002) that provide the energy to accumulate monoamine transmitters. The final catecholamine concentration gradient at equilibrium is impressive; given sufficient synthesis, vesicles can achieve levels that, if they were free in solution, would be close to a molar (Staal et al., 2004). As cytosolic catecholamine levels in chromaffin cells can reach ~10 μM (Mosharov et al., 2003), acidification provides the energy to maintain an accumulation of at least 100,000-fold.

Weak base compounds that are sufficiently membrane permeable to enter secretory vesicles bind free protons, alkalinize the existing vesicular acidic pH gradient and thus decrease the energy that drives accumulation of neurotransmitter. The alkalinization of vesicle interiors by AMPH was initially demonstrated on isolated chromaffin vesicles and in organelles of cultured midbrain DA neurons (Sulzer and Rayport, 1990). The concentration required for AMPH to collapse 50% of the chromaffin vesicle proton gradient was ~50 μM for isolated vesicles. The effect of AMPH on chromaffin vesicle pH gradients was neither stereo-specific nor blocked by reserpine, suggesting that much of its entry into the isolated vesicle preparation was due to lipophilic diffusion rather than via VMAT1. The relationship between the proton gradient and vesicular monoamine accumulation is not linear and alkalinization of lumenal pH from pH 5.6 to 5.9, a seemingly unimpressive change, would in theory lead to a loss of 75% of vesicular transmitter.

The hypothesis of vesicular pH gradient collapse by AMPH was recently confirmed in cultured chromaffin cells with improved estimates of pH using fluorescent ratiometric methods (Lee et al., 2010; Markov et al., 2008). Surprisingly, longer-term METH (24 H) can lead to a rebound acidification with an accompanying delayed increase in quantal size (Markov et al., 2008); the mechanism underlying this apparent compensation is unknown.

Even compounds such as ammonium chloride and chloroquine, agents long used to disrupt pH gradients in the laboratory, release DA from cultured DA neurons (Sulzer et al., 1993) and intact striatum as measured by microdialysis (Sulzer et al., 1992) via reverse transport following vesicle alkalinization. To date, all compounds that collapse vesicular pH gradients, including the weak bases chloroquine and ammonium chloride (Mundorf et al., 1999; Pothos et al., 2002), and vesicular chloride channel blockers and the H+-ATPase inhibitor bafilomycin (Pothos et al., 2002), lower the number of catecholamine molecules in vesicles in situ, i.e., decrease quantal size (Sulzer and Pothos, 2000), presumably by redistribution to the extravesicular milieu following pH gradient collapse.

There are, however, phenomena that occur at vesicles not explained by this action. First, there is no straightforward relationship between effects on pH gradients and monoamine accumulation, which appears mostly to be due to differential VMAT binding (Reith and Coffey, 1994). Second, the effect of pH gradient collapse on monoamine release from isolated vesicles is comparatively less efficient than that on monoamine uptake (Sulzer and Rayport, 1990); this contrasts with findings of more effective AMPH-mediated release than uptake blockade at plasma membrane transporters. This could be due to intravesicular

binding sites, such as chromogranins that bind catecholamines and decrease the genuine free concentration of transmitter in vesicles.

Several studies have tested the weak base hypothesis by comparing effects on vesicular pH and catecholamine redistribution. In isolated synaptic vesicles from whole rat brain, 3 μM AMPH depleted at least 70% of previously accumulated labeled DA, but only collapsed the proton gradient by 12% (Floor and Meng, 1996), although the level of alkalinization from higher levels of AMPH (~100 μM) appeared to correlate with release. In chromaffin granule ghosts, AMPH inhibited DA uptake more effectively than expected from its collapse of pH (Reith et al., 1993). Another indication that alkalinization may not be sufficient to fully explain redistribution of vesicular DA is that bafilomycin, a proton pump inhibitor that is not a VMAT substrate, decreased the pH gradient 2-fold more than AMPH (Floor and Meng, 1996), but released DA at only half the rate.

Perhaps most damning to the completeness of the weak base action for explaining effects at vesicles is that the (S+)-AMPH stereoisomer is several-fold more effective at blocking uptake than the (R−)isomer (Peter et al., 1994). In addition to the lack of a simple relationship between pH gradients and uptake blockade in isolated vesicles as above, these experiments endorse a role for VMAT competition, as the (S+)-isomer exhibits preferential binding to the transporter (Erickson et al., 1996; Peter et al., 1994).

VMAT substrate actions—The vesicular monoamine transporters that harness the energy from vesicle acidification to accumulate catecholamines, histamine, and serotonin were initially cloned from PC12 cells (Liu et al., 1992), and later termed the vesicular monoamine transporter 1 (VMAT1). The closely related gene expressed preferentially in the CNS is now known as VMAT2 (Erickson et al., 1992). AMPH and MDMA exhibited 10–20-fold higher affinity for VMAT2 than VMAT1 (Erickson et al., 1996). VMAT2 preferentially binds the S(+)-isomer (Gonzalez et al., 1994; Peter et al., 1994; Reith et al., 1993).

It has not been straightforward to prove that AMPH is a transported substrate of VMAT (Schuldiner et al., 1993). While uptake blockade would not itself deplete vesicular transmitter, there is an ongoing leak of vesicular transmitter, particularly with synaptic vesicles (Floor et al., 1995; Pothos et al., 2000; Schonn et al., 2003), even in the presence of the uptake inhibitor, reserpine. Moreover, as a substrate, AMPH could facilitate exchange diffusion across VMAT (Partilla et al., 2006), or cause protons to be released from the lumen via VMAT. The combination of these effects would increase cytosolic monoamines providing that AMPH in binding VMAT does not block reverse transport. Such blockade appears unlikely given a leak of vesicular transmitter with reserpine, which binds strongly to the cytosolic face of the transporter, although the mechanism by which this occurs remains unclear.

AMPH actions at plasma membrane transporters

The first identification of a specific transmitter uptake system was by Barbara Hughes and Bernard Brodie, who examined serotonin and catecholamine uptake in guinea pig blood platelets (Hughes and Brodie, 1959; Hughes et al., 1958), and Dengler and collaborators first demonstrated CNS uptake of catecholamines (Dengler et al., 1961). Further insights were provided by Brodie's lab technician, Julius Axelrod, who received a Ph.D. after twenty-one years of work in the Brodie lab (Axelrod, 2003), and then the 1970 Nobel Prize, in part for that work.

In a reformulation of Burn and Rand's earlier categories of sympathomimetics, Axelrod divided drugs that elevated norepinephrine levels in the blood into compounds that a) *prevent norepinephrine uptake*, or b) *release norepinephrine* (Axelrod et al., 1961). The

tricyclic antidepressants imipramine and chloropromazine were labeled uptake blockers, while reserpine, AMPH, and tyramine were considered releasers. Axelrod confirmed Burn and Rand's prior hypothesis that AMPH releases catecholamine, and eventually showed that AMPH blocked both uptake and release of labeled norepinephrine in brain (Axelrod, 1971), underlining the theme that both effects at the transporter may be important.

Plasma membrane and vesicular neurotransmitter transporters utilize electrochemical energy derived from ion gradients and the transmembrane electrical potential. In principle, concentrative transporters must possess at least one conformation that prevents substrates from simply diffusing down their concentration gradient, a property often labeled a *gating mechanism*. Literature on the traversal of a substrate between internal and external faces often invokes an *alternating access* model (Jardetzky, 1966), meaning that binding sites for substrates and co-substrates are alternately exposed to extracellular and cytoplasmic environments *via* conformational changes in the transporter.

Catecholamine uptake by membrane transporters required an ion gradient that would presumably act as a coupled co-substrate, principally Na^+ (Iversen, 1963), to drive accumulation of transmitter against a concentration gradient. In striatal synaptosomes, DA accumulation against a concentration gradient required the cotransport of 2 Na^+ and 1 Cl^- ions (Krueger, 1990), resulting in net import of two cations per transport cycle, and these values are now widely assumed under "physiological" conditions for DAT although they remains controversial (Pifl and Singer, 1999). The electrical gradient (i.e., membrane potential) also contributes to the driving force for substrate uptake. A variety of studies in the 1970's examined effects of AMPH stereoisomers on plasma membrane uptake transport of tritiated catecholamines by synaptosomes and most (Moore, 1978) found the S(+)-isomer more potent at blocking DA uptake by DAT.

A clear demonstration of genuine AMPH uptake in neuronal-like cells waited until 1984 (Bonisch, 1984), mostly due to background lipophilic uptake. The first convincing data that confirmed AMPH as a transporter substrate in neurons used low concentrations (5 nM) of radiolabeled AMPH and striatal synaptosomes (Zaczek et al., 1991a). They showed that AMPH accumulation was saturable, ouabain-sensitive and temperature-dependent, consistent with active transport. The DAT blockers GBR12909, methylphenidate, and cocaine, as well as METH, were potent inhibitors of AMPH accumulation. Interestingly, the lab's accompanying study showed evidence that the AMPH once accumulated into the cytoplasm may not be free but rather bound to small soluble acidic peptides in the cytosol (Zaczek et al., 1991b).

Transfection of DAT into cells with whole cell electrophysiological recordings of the cotransported currents have confirmed that AMPH is a substrate for DAT. Blockers such as cocaine inhibited inward currents due to Na^+ cotransport, while AMPH activated them (Sonders et al., 1997). This approach thus clearly identified AMPH as a substrate as it activates cotransport. It appears that AMPH binds even in the absence of extracellular Na+ in contrast with cocaine, for which Na^+ (or Li^+ substituted for Na^+) appears to be required for binding (Reith et al., 1980; Sonders et al., 1997; Wang et al., 2003).

The extent of contributions of both reverse transport and uptake blockade to AMPH action in striatum was estimated using rapid electrochemical recordings in brain striatal slice preparations and a "random walk/finite difference" analysis that incorporated Michaelis-Menten kinetics. The results showed that AMPH's effect on DA overflow was due to both reverse transport primarily but with an additional important contribution from reuptake inhibition (Schmitz et al., 2001).

The most prominent model used to explain how AMPH induces DA release at plasma membrane uptake transporters is the *facilitated exchange diffusion model* based on concepts introduced by Wilfred Stein and colleagues to describe glucose transport (Stein, 1967). The model relies on a binding site for substrate that can crisscross the plasma membrane. To accumulate cellular glucose, the binding site would take up a molecule of glucose extracellularly and then translocate the molecule across the membrane to release the glucose in the cytosol. Reverse transport would occur when the binding site faces the cytosol after release of the substrate, where it could bind another molecule of cytosolic glucose and later release it extracellularly following its re-transversal to the external site. An important aspect of this model is that the traversal of the binding site is driven by the substrate; thus, there would be a limit of no more than one molecule of glucose released from the cell for each molecule taken up. In Stein's classical formulation, transport in the reverse direction would be relatively infrequent due to sodium binding to the binding site, which would be much greater at the extracellular face and favor a confirmation that preferentially elicits uptake. This idea was specifically extended to catecholamine transporters by Brodie, who suggested that Na^+, present at higher levels on the extracellular surface, would favor an outward facing orientation (Bogdanski and Brodie, 1969).

In its adaptation to explaining AMPH action (Paton, 1973), the facilitated exchange diffusion model states that AMPH-induced DA release results from translocation of AMPH as a substrate of DAT, thus increasing the probability that the DAT binding sites face the cytosol. Then DA, which is at higher concentration in the cytosol, could bind the internalized binding site, thereby increasing the rate of reverse transport of DA. A molecule of DA released by reverse transport would follow the uptake of an AMPH molecule, and in this model's classical form, there would be at most one molecule of DA released for each molecule of AMPH accumulated. Since there is less intracellular sodium to immobilize the inward face, most of the reverse traversals of the binding sites would return empty. AMPH, on the other hand, would work by increasing the rate of outward-to-inward traversal. Cotransport of Na^+ with a substrate could increase the affinity for substrate for the inward face, and favor reverse transport (Sammet and Graefe, 1979).

One prediction that might specifically endorse facilitated exchange diffusion is that for compounds that elicit release via exchange, those that are better substrates for uptake should also be better releasers. The first study I am aware of to test Paton's theory of facilitated exchange diffusion as a means of AMPH action was by Rutledge and colleagues (Arnold et al., 1977), in which they showed that the S(+)-AMPH was a more potent releaser of DA and norepinephrine than its stereoisomer. They wrote that the temperature dependence and stereospecificity of AMPH-mediated release "suggest that a carrier-mediated, facilitated diffusion is involved in AMPH-induced transport of norepinephrine and DA."

A specific prediction of a linear relationship between uptake and release (Fischer and Cho, 1979), reported a nearly linear relationship between the inhibition of DA uptake and release for both AMPH optical isomers, and three other sympathomimetics. For instance, S(+)-AMPH was about 3-fold more efficacious than the R(−) isomer for both uptake and release. They concluded that while AMPH enhances cytoplasmic DA levels by "stimulation of granular release," i.e., redistribution of vesicular DA, and by MAO inhibition, there was no release of DA unless AMPH was taken up the transporter. This interpretation was confirmed by electrochemical experiments with cocaine and tyramine (Chen and Justice, 1998). In contrast, Trendelenburg and collaborators showed a non-linear relationship between uptake and release of various AMPH-like compounds (Langeloh et al., 1987). They did not confirm the predicted straightforward relationship between uptake and release, but rather that some compounds including AMPH were better releasers than substrates for uptake. Zinc, moreover, stimulates efflux of intracellular [^{3}H]DA despite its concomitant inhibition of

uptake (Scholze et al., 2002). Thus, there are multiple situations that that defy the prediction that efflux straightforwardly depends on whether transporter binding sites face inward or outward.

In summary, despite the difficulty in directly proving classical facilitated exchange diffusion, there is also little reason to state that it does not occur. There are instances of AMPH-driven reverse transport that facilitated exchange diffusion cannot explain, e.g., AMPH injected directly into giant DA neurons induces DA reverse transport although the AMPH was never transported by DAT (Sulzer et al., 1995). Similarly, membranophilic weak bases that are not DAT substrates, such as chloroquine and methylamine, collapse synaptic vesicle pH gradients and induce reverse transport halted by DAT blockers (Sulzer et al., 1993), while under some conditions increasing intracellular Na^+ can drive DA efflux even in the absence of extracellular AMPH (Khoshbouei et al., 2003; Raiteri et al., 1979). Thus, classical facilitated exchange diffusion is not sufficient to explain all of the release of catecholamines by AMPH.

Channel-like transporter modes—Patch clamp recordings of cell lines expressing catecholamine transporters have displayed transient very large events that may indicate an ion channel-like mode of conduction of catecholamine (Galli et al., 1996, 1998). Such events resemble uncoupled ion conductances similar to those recorded in classical ion channels as well as DAT (Sonders et al., 1997). Combining patch-clamp with amperometric recordings, Aurelio Galli's lab demonstrated that the channel-like activity of DAT was associated with transmitter flux from DA neurons that could occur through an aqueous transporter pore (Kahlig et al., 2005). The channel-like events were rare, consisting of ~10,000 molecules released over at most a few milliseconds, about the size of quantal DA release events during synaptic vesicle fusion (Pothos et al., 1998). The authors estimated that about 10% of AMPH-mediated DA released was due to the channel-like events. More strikingly, extracellular AMPH increased the frequency of channel-like release events by ~8-fold, while extracellular DA had no effect on the frequency of the channel-like events.

The ability of AMPH, membranophilic weak bases, and ions to release transmitter even when not taken up by DAT, as well as the demonstration of AMPH-induced channel-like release events, indicates that a more detailed explanation of structural changes that occur during reverse transport is required. An aspect may be due to AMPH's ability to stimulate the co-transport of ions, and the consequent rearrangement of electrochemical gradients (Khoshbouei et al., 2003; Sitte et al., 1998). The idea that AMPH's increase of intracellular Na^+ concentration may be sufficient to stimulate AMPH-induced DAT-mediated DA efflux (Khoshbouei et al., 2003) has been used to propose that this action is essential for its stimulation of DA efflux (Pifl et al., 2004; Pifl and Singer, 1999). This notion may be consistent with a "unified" model that encompasses roles for both substrate and ion gradients, as well as channel-like properties in which multiple substrates could be transported without a shuttling binding site, as suggested for bacterial transporters (Abramson et al., 2003; Huang et al., 2003) in a *rocker-switch alternating access* model, in which tilting of transporter domains *close* a "cavity" on the cytoplasmic side (the *inward-facing conformation*) to *open* a new "cavity" on the extracellular side (the *outward-facing conformation*) (Huang et al., 2003; Locher et al., 2003). A putative glutamate transporter (Yernool et al., 2004) suggested an alternate model in which coordinated "flipper" movements of two sets of two hairpin loops allows alternating access by occluding the transporter, providing an "open state" on either side of the membrane without an "open channel" (Kavanaugh, 2004). These models introduce variations on classical facilitated exchange diffusion, and in that they do not require a binding site that traverses the membrane, they provide both for channel-like events and do not require a maximum limit of

a one-for-one molecule exchange of cytosolic substrate for extracellular AMPH during reverse transport.

Recent data (Khoshbouei et al., 2004) seem consistent with such a *unified model*, invoking an asymmetric transporter that suggests a conformational property of DAT that typically favors influx over efflux, but with net flux controlled by transmembrane substrate gradients, and introduces a potential second messenger system that may provide the basis for these observations. Evidence for an asymmetric conformation of the transporter is that when the first 22 amino acids of the N-terminal region of DAT were truncated or if serine residues in that region were mutated to alanine (which cannot be phosphorylated), AMPH-mediated DA efflux was reduced by ~80%. As mutating the same serine residues to aspartate in order to simulate serine phosphorylation resulted in normal AMPH-mediated efflux, the authors suggested that phosphorylation of serines may shift DAT from a “reluctant” state to a “willing” state that favors AMPH-induced DA efflux without disturbing normal DA uptake.

DAT features numerous putative phosphorylation sites and multiple protein kinases have been found to regulate DAT function (Carvelli et al., 2002; Granas et al., 2003; Loder and Melikian, 2003; Melikian and Buckley, 1999), some likely by membrane trafficking and endocytosis. AMPH can increase protein kinase C (PKC) activity (Giambalvo, 1992) which can stimulate DAT-mediated release of DA (Giambalvo, 1992). A clue to effects of DAT phosphorylation may be that Ca^{2+}/calmodulin-dependent protein kinase–II (CaMKII) has been implicated in the ability of AMPH to cause DA reverse transport (Fog et al., 2006), possibly via promoting a linkage to the SNARE protein, syntaxin-1 (Binda et al., 2008). This action of syntaxin-1 is suggested to potentiate channel like activity at DAT (Carvelli et al., 2008). A new and interesting model is that AMPH, by enhancing Na^+ and Ca^{2+} influx, activates PKC and CAMKII, which phosphorylates DAT, favoring a syntaxn 1 association that stimulates the channel mode of reverse transport (Robertson et al., 2009).

Thus, there are multiple suggestions to how a combination of AMPH and its influence on phosphorylation could elicit a DAT conformation that favors DA efflux. This would also be a non-classical variant of facilitated exchange diffusion without a requirement of a one-for-one exchange of DA and AMPH molecules.

DAT activation by AMPH can under some conditions even induce sufficient excitatory current to potentiate neuronal firing (Ingram et al., 2002), which could explain reports of AMPH-induced neuronal excitation (Shi et al., 2000) and exocytic catecholamine release (Darracq et al., 2001; Pierce and Kalivas, 1997).

AMPH actions on DA synthesis and degradation—AMPH is a competitive inhibitor of MAOs, mediators of amine catabolism on the outer mitochondrial membrane, but it is not degraded by the enzyme. AMPH shows five-fold or greater selectivity for MAO A over MAO B, with an affinity for MAO A in the range of 10 μM with the S(+) enantiomer, (Mantle et al., 1976; Robinson, 1985), similar to the preference for S(+)-AMPH by DAT and VMAT. AMPH is thus likely concentrated in the cytoplasm to a level that inhibits MAOs, a major metabolite of AMPH. An AMPH metabolite, 4-OH-AMPH (α-methyl-*p*-tyramine), is moreover likely to serve as a competitive inhibitor of MAO A (Cho and Kumagai, 1994).

AMPH has long been noted to enhance DA synthesis, and this provides an important role in its action under some conditions, e.g., following reserpine treatment. This effect can be so profound that AMPH released far more DA into the neuronal culture medium than was present in the entire culture prior to AMPH exposure (Larsen et al., 2002). In striatal synaptosomes, AMPH at concentrations up to 15 μM enhanced DA synthesis by as much as

70% (Fung and Uretsky, 1982; Kuczenski, 1975). The mechanism by which AMPH enhances TH activity is unknown, but could involve Ca^{2+}-dependent phosphorylation of serine residues on TH that regulate enzymatic activity (Griffiths and Marley, 2001).

Summary of effect on DA transmission—Together, AMPH possesses a variety of seemingly independent actions that make it uniquely able to enhance DA release independently of vesicle exocytosis – in fact it seems to decrease exocytic release due to its weak base properties, VMAT blockade, and activation of D2 receptor feedback inhibition.

The level of DA following AMPH exposure in the striatum probably reaches 500 nM to 10 μM (Wieczorek and Kruk, 1994) under conditions where evoked DA release is attenuated or lost (Jones et al., 1998; Schmitz et al., 2001). Thus, AMPH induces DA release in a manner uncoupled from normal salient behavior, and might be expected to enhance learned behaviors associated with self-administration.

Cocaine

Cocaine, derived (Gaedcke, 1855) from the Andean *Erythoxylon coca* and related species, was clearly identified as a sympathomimetic drug that increased blood pressure (Frolich and Loewi, 1910). Surprisingly, however, it blocked the effect of the paradigmatic sympathomimetic drug, tyramine (Tainter and Chang, 1927), and odd finding that was sometimes referred to as the *cocaine paradox*. The paradox was solved by Burn and Rand (Burn and Rand, 1958), who showed that “the action of cocaine may be to arrest the release of the noradrenaline-like substance from the store.” In other words, their study introduced the oft-confirmed finding that uptake blockers like cocaine also block reverse transport due to releasers like AMPH and tyramine. The finding that cocaine blocks AMPH-mediated release was replicated later in the CNS (Fischer and Cho, 1979; Heikkila et al., 1975).

Axelrod’s group, in a study to identify which psychostimulants block reuptake and which induce release, reported that cocaine blocked norepinephrine uptake into the nerves innervating heart, spleen, and adrenal gland (Whitby et al., 1960). To our knowledge, this study was the first to show that cocaine blocks catecholamine reuptake, now acknowledged to be its primary means of elevating extracellular catecholamine levels. Evidence for this includes that self-administration of cocaine-like blockers correlated with their binding efficacy to DAT (but not with the DA releaser, AMPH) (Ritz et al., 1987).

A contemporary means to confirm this mechanism of action has been to examine DAT deficient mice. These mice demonstrated as much as 100-fold longer enhanced levels of DA following electrical stimulation of the striatum (Giros et al., 1996) and were “indifferent” to cocaine and AMPH with no effect on locomotor activity. Surprisingly, however, they could still learn self-administration (Rocha et al., 1998), apparently as cocaine would block DA reuptake by serotonin and norepinephrine transporters (Rocha, 2003), implicating a form of compensation in those lines. A mutant mouse line with a cocaine-insensitive DAT however did not learn cocaine reward (Chen et al., 2006). Thus, it appears that the addictive qualities of cocaine are normally dependent on blockade of DAT function.

The DAT blocker nomifensine, often used to model effects of cocaine, increases the maximal amplitude of striatal DA following a single pulse from ~ 20 nM to ~ 250 nM and the decay time constant by 20-fold (Benoit-Marand et al., 2000). Thus, the effects of cocaine on neurotranmission as a blocker of normal reuptake ought to rely on evoked DA release, in contrast to that of AMPH which inhibits evoked release via decreased quantal size. A net effect of both is to indirectly activate D2 receptors, which depresses evoked release (Brodie and Dunwiddie, 1990; Schmitz et al., 2002).

How might cocaine's blockade of reuptake interact with the signaling from burst firing evoked by environmental cues versus the ongoing release due to tonic activity? These questions have become addressable recently by the introduction of means to record striatal DA during behavior by Mark Wightman and Regina Carelli. In these recordings, DA transients, presumably due to burst firing, can be measured (Wightman et al., 2007). With cocaine, the DA transients not only reach higher neurotransmitter concentrations in the striatum and nAc, which would be expected from higher levels reached due to reuptake blockade at the electrode, but also increase in frequency (Heien et al., 2005). While the means by which the number of transient release events in enhanced by cocaine is unknown, it appears to be mediated by a circuit that involves endocannabioids (Cheer et al., 2007).

A model of cocaine's effect on reuptake blockade effect indicates that diffusion will be enhanced so that far more DA receptors at sites distal from the site of transmitter release will be activated (Venton et al., 2003). This conclusion however is complicated by the observation that cocaine also increases steady-state DA (Heien et al., 2005), likely mostly due to reuptake blockade of DA released during tonic activity.

Summary of effect on DA transmission—Present results indicate that cocaine enhances DA transmission associated with both tonic and phasic activity, which should disrupt normal signaling by enhancing baseline DA levels and decoupling the relative contributions of tonic and burst firing-mediated modes. Its effect on enhancing transients may further contribute to an association of cue with reward (the same is true for ethanol and nicotine). Its mechanism of action leads to different consequences than AMPH, and self-administration may be due to disruption of the relative response to cue, enhanced tonic release, or both.

Ethanol

Ethanol is the most widely used addictive drug, imbibed by over 51% of Americans over the age of 12, with about one quarter of the population participating in binge drinking (oas.samhsa.gov). It is moreover freely imbibed by other primates (Schwandt et al., 2010), rodents (Griffin et al., 2007) and songbirds (Fitzgerald et al., 1990). Voluntary drinking releases DA in humans (Boileau et al., 2003), with recent human PET imaging showing that the equivalent of 3 drinks increased extrasynaptic DA in the striatum by 138% in men and 69% in women (Urban et al., 2010).

It is thus startling that we do not know how ethanol enhances DA transmission. One reason is because there are many possible receptor and channel targets for ethanol, as recently reviewed (Melis et al., 2009; Morikawa and Morrisett, 2010), but none that clearly show sufficient effect on DA release at levels achieved by individuals who consume alcohol for its reinforcing properties.

How many are enough?—Ethanol is typically measured as blood fraction (blood alcohol content BAC, in the US in units of g/100 ml written as a percentage). As there appears to be no blood/brain barrier to ethanol penetration, the extracellular levels in brain are close to those in blood (Robinson et al., 2002). A glass of wine (150 ml, 2.6 M ethanol) yields 0.02% BAC (4.8 mM) in blood of non-alcoholic 68 kg men, with women achieving 34% higher levels (Frezza et al., 1990). A typical level for legal intoxication is 0.08% BAC (17 mM), stupor occurs at 0.25% (54 mM), blackout at 0.35% (76 mM), and lethality at 0.4% (87 mM). Effects on DA transmission related to self-administration for reinforcing properties should therefore be present at 5–20 mM ethanol, lower than that often studied experimentally. Note however that alcoholics develop tolerance and can achieve extraordinary levels, as high as 1.20% BAC (260 mM) (Brick and Erickson, 2009).

How does ethanol cause DA release?—It has been suggested that alcohol activates VTA neurons directly to release DA into nAc. However, some but not all dialysis experiments show that alcohol application into the nAc alone locally increases DA overflow while application of alcohol into the VTA does not (Ericson et al., 2003; Yim et al., 1998), suggesting that effects at the axons may be required. In contrast, studies of ethanol using cyclic voltammetry in the striatal slice (Budygin et al., 2001) demonstrated effects of ethanol on evoked DA release only at very high (100–200 mM) alcohol levels, which depressed release. Thus, the relevant brain sites in striatum are still unclear.

Ethanol might increase DA release by direct excitation of DA neurons. Some studies show an effect of alcohol to excite DA neurons in the VTA at concentrations of 20–320 mM (Brodie et al., 1990; Okamoto et al., 2006). This might involve an inhibition of potassium channels, including those that regulate after-hyperpolarizations and the rate of burst firing, as well as sustained K+ currents (Koyama et al., 2007).

Alternatively, ethanol may act via disinhibition of DA neurons, most likely at GABA receptors. Ethanol effects on GABA are suspected to play a part in its effects, notably the motor-impairing and anxiolytic responses, in a manner related to the benzodiazepines and barbiturates. Recent evidence suggests a possible role for extrasynaptic GABAA receptors, as a population of extra-synaptic receptors containing α-4 subunits were found that provide a steady inhibition of thalamic neurons with sedative levels (50 mM) ethanol, (Jia et al., 2008). A recent paper showed that viral knockdown of the α-4 subunit in the nAc shell but not the core, decreased alcohol drinking and preference in the rat (Rewal et al., 2009); perhaps the receptors in that area are more sensitive than those in the thalamus.

Several classes of striatal interneurons exert “veto” power on the ability of MSN neurons to fire (Tepper et al., 2004) and if either these or medium spiny neuron collaterals were inhibited, the net result of ethanol inhibition of GABAergic activity (or cholinergic) activity via GABAergic disinhibition could underlie a presynaptic component of enhanced DA release.

A similar pathway mediated by an ethanol-mediated GABA receptor disinhibition of DA neurons by ethanol could also occur in the ventral midbrain (Mereu and Gessa, 1985), as found with opioids and sedatives (see below). It should be noted that, in contrast, ethanol appears to enhance GABAB currents on midbrain DA neurons by activating GIRK currents (Federici et al., 2009), which may reinstate some inhibition. Alternatively, opiate receptors on GABAergic VTA projection neurons have been implicated in the action of ethanol (Xiao and Ye, 2008), perhaps via ethanol-mediated release of β-endorphin, which would similarly disinhibit DA neurons.

Other hypotheses have been offered for network effects via cannabinoids, serotoinin, glycine, NMDA channels, nAChR, and NMDA receptors, but to date none have been shown to clearly cause enhanced firing of DA neurons at levels of 5–20 mM ethanol, although the enhancement of burst firing seems to involve endocannabioids (Cheer et al., 2007). In summary, while there is good evidence that levels of ethanol achieved during moderate drinking release DA, and while there are a multitude of potential targets, the means by which ethanol releases DA during drinking at nominally rewarding levels remain unclear.

Acetaldehyde hypothesis—An alternate idea that has been percolating for decades is that the active agent responsible for DA release is actually a metabolite. One so studied was an endogenous opioid, tetrahydropapaveroline, a DA product that is enhanced by ethanol (Goldstein and Judson, 1971; Walsh et al., 1970).

Another is the alcohol dehydrogenase product, acetaldehyde, which is volatile and difficult to handle experimentally. Nevertheless, Melis and colleagues have found that acetaldehyde increases midbrain DA neuron firing via a decrease of potassium current and activation of hyperpolarization-activated inward currents (Melis et al., 2007). As mice will self-administer acetaldehyde as well as ethanol (Melis et al., 2009), it is possible that this ethanol metabolite, which is highly reactive and thought to underlie liver toxicity in alcoholism, may be responsible for DA release. If true, it may help explain the mystery of why levels of ethanol that enhance DA release in vivo are ineffective in the slice: because there is a slower rate of alcohol metabolism than in the animal.

While there are studies suggesting that tonic firing rates could be altered by ethanol via effects on potassium channels, current in vivo data appears to endorse an increase in DA release from phasic firing. Using intravenous administration to remove the effects of cues and cyclic voltammetry in the nAc of awake rats, Robinson and colleagues discovered small subregions that reproducibly respond to intravenous ethanol at doses of 0.125 g/kg and higher with increased DA transients associated with burst firing (Robinson et al., 2009). The authors conclude that there is regional variability within the NAc at a “micro” level and that this could be due to groups of DA neurons with overlapping burst activity (Robinson et al., 2009). Alternatively, increased DA transients could be due to presynaptic effects on release probability, as with the interneuron modulation suggested above.

If so, ethanol may have very different effects on DA neurotransmission than AMPH and cocaine, which are independent of activity and effective at enhancing signal from both tonic and phasic cues, respectively, in that ethanol may specifically enhance the signal from salient cues but have little effect on DA levels associated with tonic activity.

Opioids

The initial modern western scientific monography on opium suggested that it acts at membranes, allowing vessels and nerves to expand (Jones, 1701). Specific binding sites, the opiate receptors, were identified using radiolabeled opioids somewhat later (Goldstein et al., 1971), and were found to encompass multiple subtypes.

Rats will self-administer opioids to the VTA (Bozarth and Wise, 1983), and the use of relatively specific ligands indicated that reinforcement was mostly due to the μ opiate receptor (Devine and Wise, 1994; Di Chiara and Imperato, 1988b). Consistently, μ opiate receptor deficient mice do not show opioid-mediated place preference learning, but continued to develop place preference for cocaine (Contarino et al., 2002).

Most efforts to study opioid effects are on DA neuron firing: however, mice will self-administer opiates to the nAc as well (David and Cazala, 2000), and as detailed below, opiates can provide a frequency-dependent filter of DA transmission via a circuit including ACh.

Effects on DA neuron firing via disinhibiton—Opioids increase burst firing of VTA DA neurons (Gysling and Wang, 1983; Nowycky et al., 1978). The second set of authors suggested that opioids, by acting to depress activity of local presumably GABAergic neurons in the VTA, or modulating striatonigral inputs to the SN, would interfere with their recently characterized inhibition of DA neuron activity (Grace and Bunney, 1979).

This opioid disinhibition hypothesis was strongly endorsed by Johnson and North (1992), who used opiate receptor ligands to confirm that hyperpolarization of the GABA neurons was due to the μ receptor, while agonists of that receptor had no direct effects on the DA neuron. Importantly, opioids that acted on the GABA neurons inhibited inhibitory synaptic

potentials on the DA neurons: thus, it appeared that opioids act at μ receptors on local GABA interneurons, hyperpolarizing them and inhibiting GABA release, and in turn disinhibiting DA neurons.

It should be noted that these effects may not chiefly be GABA interneurons but rather collaterals of projection neurons (Omelchenko and Sesack, 2009; Steffensen et al., 1998), and may include effects on GABAergic efferent neurons, for instance from the striatum or laterodorsal tegmentum (Sesack and Grace, 2010; Tepper et al., 1995).

Effects on release probability from terminals via an ACh loop—Using cyclic voltammetry in the striatum, Britt and McGehee found that opioids modulate release probability in a stimulus-frequency dependent manner (Britt and McGehee, 2008). In the striatal slice, opioid μ receptors inhibit DA release from single electrical pulses, but have relatively little effect on release from stimuli meant to emulate burst firing, due to presynaptic facilitation during bursts.

During presynaptic facilitation, the amount of transmitter released by subsequent stimuli at short intervals is augmented by a first (priming) stimulus because the increased Ca^{2+} from each stimulus has a decay time of a few hundred milliseconds, and presynaptic Ca^{2+} levels reach higher levels during the subsequent stimuli. Presynaptic facilitation in response to burst stimuli is also observed with nicotine (see below), and ACh is involved in the effects of opioids on striatal DA release. DA terminals do not have μ receptors (Trovero et al., 1990), while striatal large tonically active neurons (TANs) that release ACh do express these receptors. μ opioid agonists decrease the firing of TAN neurons, which decreases ACh release, and thus the probability of synaptic vesicle fusion, as release probability in DA terminals is enhanced by the ambient ACh activation of presynaptic nAChR receptors (see below). This inhibition is however overcome by presynaptic facilitation of release due to buildup of higher levels of presynaptic Ca^{2+} that occur in the DA terminals during higher frequency activation of voltage-gated Ca^{2+} channels (Zhang and Sulzer, 2004).

As with ethanol and cocaine, the enhancement of burst firing by opioids appears to involve endocannabinoids, although the means by which this occurs is unclear (Cheer et al., 2007).

Summary of effect on DA transmission—Thus, opioids, by increasing DA release by stimulating burst firing, while selectively inhibiting release from tonic firing via actions at the terminals, will enhance the magnitude of phasic DA neurotransmission relative to baseline, which would be expected to enhance salience and perhaps the learning associated with reward and addiction.

Sedatives

With all of these drugs, there has been argument about the potential for addiction, and the controversy about sedatives continues. Benzodiazepines are said to be mostly taken by abusers to augment the effects of a “primary” addictive drug such as methadone (O’Brien, 2005). Currently, between 10–42% of seniors use benzodiazepines prescribed for anxiety and sleep disorders (Voyer et al., 2010). After several weeks of administration the drugs are ineffective, and it is speculated that continuing self-administration is due to a “psychological dependence” that might be differently parsed from addiction.

Barbiturates, introduced in 1903 (Fischer and v Mering, 1903), were used in epidemics in the 1930s and 40’s, with 14% of admissions for ten major hospitals for barbiturate abuse (Cozanitis, 2004). Since the introduction of benzodiazepines, their use as sedatives has fallen, although they are still widely used as anitconvulsants, and due to decreased availability to patients, there is relatively little current abuse.

Barbiturates are agonists of GABAA receptors, and at high concentrations (>50 μM) can open the ion channel in the absence of GABA (D'Hulst et al., 2009). In contrast, benzodiazepines, which bind at the interface of α and γ subunits, are "allosteric agonists" that enhance the frequency of ion channel opening when GABA is also bound. Unlike ethanol, which binds with higher affinity to GABAA receptors that contain the α4 subunit (see above), benzodiazepines preferentially bind and facilitate current at receptors with the α1 subunit (Pritchett et al., 1989).

Effects on DA neuron firing—Midbrain DA neurons lack α1 subunit isoforms (Okada et al., 2004) while many GABA neurons express this subunit (Fritschy and Mohler, 1995). Recently Luscher and colleagues found that while the benzodiazepine midazolam (MDZ) enhanced inhibitory currents measured at DA neurons and fewer spikes were generated, this response was absent in mice that possess a mutation in the α1 receptor site responsible for binding benzodiazepines (Luscher and Huber, 2010). As expected, wild-type GABA neurons also showed a decrease in firing with MDZ, but this was absent in the mutant line, while the neurons continued to show reduced firing in response to morphine. The mutant line further did not learn to self-administer MDZ. Thus, as above with opioids, and possibly ethanol, benzodiazepines enhance DA neuron firing via disinhibition, although they rely on different means to inhibit GABA neurons, in this case due to preferential binding to α1 containing receptors.

Phenobarbital has long been known to release DA (Di Chiara and Imperato, 1986), although to my knowledge no studies have addressed the neuronal circuitry by which this occurs.

Effects on release probability from terminals—To my knowledge, the effects of barbiturates and benzodiazepines on locally evoked striatal DA neurotransmission have not been examined directly, although we find that GABAa agonists and antagonists have profound effects on evoked DA release (Hui Zhang and D.S., unpublished results). In striatal slice experiments using cyclic voltammetry, the GABAA receptor agonist muscimol (100 μM, 4 min) inhibits evoked DA release, while the antagonist, GABAzine (10 μM) had no effect, indicating that tonic GABA in the slice is insufficient to cause ongoing inhibition of DA release. Whether the effect of musicmol is directly on DA neuron terminals or exerts its effect via a circuit, as do opioids, is unknown.

Similar to effects with opioids and nicotine, the inhibition is significantly greater for single pulse stimuli than for stimuli that emulate burst firing: perhaps this is a general property of drugs that act via disinhibition.

Nicotine

The prevalence of nicotine self-administration is second only to ethanol, with a third of the population over age 12 using tobacco products in 2009 (oas.samhsa.gov). It is the largest cause of death in the US (18.5%), at 25-fold greater levels than all illicit drugs combined, and 5-fold greater than deaths related to alcohol (Mokdad et al., 2004): one must ask if we are selecting the appropriate drugs for prohibition. Nicotine is not reported to induce long-term tolerance to its rewarding effects, although it produces tolerance to its aversive properties as well as receptor desensitization, and so may not quite fulfill the Awsiter-derived criteria for drugs of addiction in comparison to the others discussed.

Nicotine's mechanism of enhancing DA release is to date unique for addictive drugs in that it seems to mostly be due to enhancing excitatory input to DA neurons via presynaptic activation: however, there is additionally evidence for disinhibition analogous to effects of opioids and sedatives, a direct effect on DA cell bodies, and altered filtering due to

presynaptic receptors on DA terminals. These relatively complex actions have been recently reviewed (Dani and Bertrand, 2007; Mao and McGehee, 2010; Placzek et al., 2009).

The pharmacologically relevant level of nicotine and the composition of nAChR at particular sites is important due to different kinetics of activation, desensitization, resensitization, and upregulation. Maximal brain free nicotine concentration is estimated as 240 nM per cigarette, which requires 2–3 min, declining to 25 nM overnight (Rose et al., 2010).

α7 receptors are homomeric pentamers that are highly permeable to Ca^{2+}, and exhibit a relatively low affinity for nicotine and are thought to not desensitize during smoking. α4β2 pentamers have high (1.6 μM ACh) and low affinity (62 μM ACh) states, with prolonged nicotine increasing the high affinity contribution and also desensitizing these receptors at reinforcement-related nicotine levels (Dani and Bertrand, 2007).

Effects on DA neuron firing—Identification of the precise nicotinic receptors on midbrain DA neurons relies on pharmacological tools and remains incompletely characterized. The receptors are expressed on both VTA than SN DA neurons (Nashmi et al., 2007), and include α7 homomers and α4 and α6 subunits associated with β2. While these receive a relatively low direct excitation from ACh, as glutamate antagonists block all spontaneous excitation in VTA neurons (Mao and McGehee, 2010), nicotine, however, can directly increase firing of VTA neurons (Schilstrom et al., 2003) via β2 receptors (Picciotto et al., 1998), which desensitize after a few minutes. This led John Dani and collaborators to suggest that desensitization of the receptors is “a cellular basis for reports that the first cigarette of the day is the most pleasurable” (Pidoplichko et al., 1997).

AChR desensitization on additional neurons also plays key roles. Nicotinic receptors on VTA GABAergic neurons are mostly α4/β2, although there are apparently some α7 currents. Self-administered levels of nicotine first activate and then desensitize these receptors, leading to disinhibition of DA neurons (Mansvelder et al., 2002), similar to the effects of opioids and sedatives discussed abobve.

More particular to nicotine, however, is the role played at glutamatergic inputs to the ventral midbrain DA neurons, which have presynaptic α-7 receptors that do not desensitize during smoking. These presynaptic receptors provide Ca^{2+} entry and thus enhance glutamate release. Therefore, while the GABA receptors rapidly disinhbit DA neurons, the presynaptic inputs increase activity, leading to enhanced burst firing. An additional factor, that may apply to other addictive drugs (refer to *Malenka and Luscher* review in this issue) is that this leads to long term potentiation so rapidly that it may occur even while an individual is smoking a cigarette (in contrast to for example administraton of cocaine or opioids), leading to even more burst firing and associated DA release (Mansvelder et al., 2003).

Effects on release probability at DA terminals—Nicotine perfused directly into NAc also enhances local DA release, presumably by a presynaptic action on the DA terminals of this region (Westfall et al., 1988). Normal cholinergic release in the striatum is due to TANs that are small in number (~ 1% of striatal neurons) but probably interact with all striatal neurons (Zhou et al., 2001). This tonic release activates presynaptic nicotinic and M5 muscarinic receptors on DA neurons, which help to maintain DA neurons in a state of higher release probability (Bendor et al., 2010; Zhou et al., 2001).

The presynaptic nicotinic receptors on DA terminals possess β2 subunits (Salminen et al., 2004; Zhou et al., 2001) that desensitize rapidly with nicotine. As normal tonic ACh release provides nAChR activation that maintain DA terminals in a state of higher release

probability, nAChR antagonists inhibit release. However, as with the DA cell bodies, nicotine desensitizes the receptors, providing the odd phenomenon that both nicotinic agonists and antagonists inhibit evoked DA release following single stimuli. This inhibition, via either antagonists or desensitization, is overcome during higher frequency stimuli meant to emulate burst firing (Rice and Cragg, 2004; Zhang and Sulzer, 2004), as the probability of release is enhanced by Ca^{2+} entry during presynaptic facilitation (see above).

Summary of effect on DA transmission—Nicotine, by enhancing burst firing at DA cell bodies via disinhibition and presynaptic excitation, while accentuating DA release from burst firing and filtering release during tonic firing, would be expected to profoundly affect the magnitude of phasic DA neurotransmission relative to baseline, which would enhance the response to cues associated with self-administration.

Solvents

A particularly ghastly addiction is the use of glue as an inhalant, a practice that costs a few cents and is routinely performed by millions of homeless children in Africa, Asia, South America and the Middle East. In Sao Paulo, Brazil, for example 77.5% of homeless children from age 6–17 use solvents (Kozel et al., 1995). Anecdotally, many of these children do not survive, and chronic ingestion may lead to renal failure (Saxena and Ul-Haq, 2005) as well as neuropathy. Oddly, in southeast Asia, this drug is often known as *dendrite* in Nepal after an adhesive manufacturer (Thapa et al., 2009). While now uncommon, there was a related epidemic of ether addiction in 19th century Ireland as a whiskey substitute (Hart, 1890) that reappeared in Brazil in the 1960s (Kozel et al., 1995).

The most studied active agent in the glues is toluene, which is thought to be the solvent most preferred by homeless children. Toluene, cyclohexane, and benzene are each self-administered by rodents (Bespalov et al., 2003), and additional small organic compounds in petrol are likely responsible for similar effects.

A microdialysis study in rats demonstrates that toluene increases DA levels in the nAc (Riegel et al., 2007). While the means by which it does so are unknown, there is some suggestion that it may be related to enhancing Ca^{2+} levels, and it may elicit a direct enhancement of presynaptic DA release, which would be a unique mechanism to date, or work to exacerbate excitation in a manner similar to nicotine by enhancing excitatory drive. The possibility of a direct excitation of DA neurons is suggested in that toluene can increase the number of quantal release events from PC12 cells, consistent with a role in enhancing Ca^{2+} currents (Westerink and Vijverberg, 2002). Both possibilities are consistent with findings that toluene and other abused inhalants enhance quantal release of GABA in the hippocampus by increasing presynaptic Ca^{2+} release from intracellular stores (MacIver, 2009).

There are also reports of toluene effects that may cause enhanced release via actions on NMDA or GABA receptors (Bale et al., 2005; Beckstead et al., 2000; Cruz et al., 1998), which could be similar to NMDA activation by phencyclidine (PCP).

8. Summary

There appear to be four major mechanisms by which addictive drugs work to reinforce self-administration, with some drugs participating by multiple means. These are 1) increasing DA neuron firing via increased excitation and/or disinhibition, 2) enhancing the relative DA transmission associated with burst firing by inhibiting release associated with tonic firing, 3) blockade of DAT reuptake, 4) increasing cytosolic DA levels while stimulating reverse transport.

It may be that additional direct effects on DA neuron firing or direct enhancement of release probability occur, as suggested by initial research with solvent drugs.

As - with the exception of the fourth mechanism - these mechanisms are required for normal learning in addition to the “diseased” learning associated with addiction, future pharmacologists and physiologists will need to be creative to design effective strategies for drug treatment that go beyond substituting one drug that exacerbates DA release for another, as already recommended by the earliest paper on drug habit (Awsiter, 1763). Improved behavioral approaches to treat addiction are certainly still well worth developing concurrently with improved understanding of drug actions.

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Table I

Acute effects of addictive drugs on DA neurotransmission

	direct actions that alters DA transmission	**relevant site**	**resulting action**
Amphetamines	DAT substrate VMAT substrate MAO inhibitor TH activator collapse of vesicular pH gradient	DA neuron somatorendritic regions: DA neuron presynaptic terminals	increased stimulation-independent DA release by reverse transport through DAT
Cocaine	DAT blocker	DA neuron somatorendritic regions: DA neuron presynaptic terminals	Enhances DA levels associated with tonic and burst firing
Ethanol	unclear, possibly disinhibition	unclear	Enhanced DA neuron burst firing
Nicotine	nAChR agonist	presynaptic excitatory and inhibitory inputs to DA neuron: DA neuron somatodendritic regions: DA neuron presynaptic terminals	Disinhibition and enhanced excitation leads to DA neuron burst firing; enhanced fraction of DA release associated with bursts
Opioids	μ opioid receptor agonists	striatal ACh neurons; ventral midbrain GABAergic neurons and axons; striatal GABAergic neurons	Disinhibition leads to DA neuron burst firing; enhanced fraction of DA release associated with bursts
Sedatives	GABAa receptor coactivators benzodiazepines)/agonists (barbtuates)	ventral midbrain GABAergic neurons and likely axons; unclear striatal sites	Disinhibition leads to DA neuron burst firing; likely enhanced fraction of DA release associated with bursts
Solvent Inhalants	unclear, may enhance release probability	unclear	unclear

DAT, plasma membrane DA uptake transporter; MAO, monoamine oxidase; nAChR, nicotinic acetylcholine receptor; TH, tyrosine hydroxylase,