The Neurobiology of Opiate Motivation

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Opiates are a highly addictive class of drugs that have been reported to possess both dopamine-dependent and dopamine-independent rewarding properties. The search for how, if at all, these distinct mechanisms of motivation are related is of great interest in drug addiction research. Recent electrophysiological, molecular, and behavioral work has greatly improved our understanding of this process. In particular, the signaling properties of GABA_A receptors located on GABA neurons in the ventral tegmental area (VTA) appear to be crucial to understanding the interplay between dopamine-dependent and dopamine-independent mechanisms of opiate motivation.

Opioid drugs play an integral part in human society, in part because of their well-documented analgesic properties (Kanjhan 1995; Rosenblum et al. 2008). However, many of these compounds—for example, heroin and other opiates—are among the most addictive drugs in human history (Kalant 1997; Gruber et al. 2007). For these and other reasons, the elucidation of their motivational mechanisms of action has remained a priority for many researchers. The study of opioids and their addictive properties, and the anatomical and biological mechanisms that underlie them, has produced a slew of data implicating various receptor subtypes and neurotransmitters. In this article, we focus on research implicating the ventral tegmental area (VTA) as a crucial locus in opiate addiction. More specifically, we review research suggesting that the biology of this brain region

appears crucial for the selection of either dopamine-dependent or dopamine-independent pathways of opiate motivation.

OPIATE REWARD AND REINFORCEMENT IN THE BRAIN

The concept of *motivation* is crucial to the survival of all animals. In broad terms, motivation can be defined as the driving force responsible for approach or avoidance behavior. For our purposes, the term *reward* describes those entities that instigate approach behavior (such as bar pressing for sucrose or an attraction to a food-paired environment). The desire to reexperience this reward can lead to increased repetitions of these behaviors, that is, “reinforcement.” Although the reinforcement of “good” behaviors such as eating is beneficial, drugs of

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abuse (such as opiates) are problematic in that they encourage detrimental drug-seeking and drug-taking behaviors.

The biological mechanisms underlying opiate reward and reinforcement have been studied in great detail. Opiates mediate their reinforcing actions via a family of G-protein-coupled receptors, which are commonly classified into major subtypes designated μ , δ , κ , and orphaninFQ/nociceptin (Minami and Satoh 1995; von Zastrow 2004; Le Merrer et al. 2009). It is their binding to these receptors—the μ (and to a lesser extent δ) opioid subtype, in particular—that is thought to be responsible for their positive reinforcing properties (Gruber et al. 2007; Le Merrer et al. 2009). Although these receptors are found in a large number of brain structures (Mansour et al. 1988; Le Merrer et al. 2009), the direct reinforcing effects of opioids appear to be limited to a select number of brain areas. Chief among these is the midbrain VTA (Wise 1989), which houses the dopamine cell bodies that project to the nucleus accumbens (NAc) and which are thought to be crucial for both natural and drug motivated behaviors (Wise and Rompre 1989; Wise 1996). Direct injection of morphine or other opioid drugs into the VTA produces robust reinforcing effects as measured by several behavioral paradigms including self-administration and place conditioning (Wise 1989; Nader and van der Kooy 1997; Olmstead and Franklin 1997; Shippenberg and Elmer 1998; McBride et al. 1999; van Ree et al. 1999).

THE VTA AND DOPAMINE-DEPENDENT REINFORCEMENT

Owing to its increased activity in response to both natural and drug reinforcers (Rolls et al. 1974; Yokel and Wise 1975; Wise et al. 1978; Spyraiki et al. 1982; Bozarth and Wise 1984; Di Chiara and Imperato 1985; Zito et al. 1985), dopamine neurotransmission has long been the focus of many theories of motivation. It is therefore unsurprising that a predominant role for dopamine in opiate motivation has endured throughout the years (Bozarth and Wise 1981; Wise 1996; Ford et al. 2006). Indeed, as discussed above, direct infusions of opiates or opi-

oid-like drugs into the VTA result in increased dopamine activity, supporting the validity of such a model (Gysling and Wang 1983; Johnson and North 1992).

However, a growing number of publications have found evidence for an alternative idea: namely, that dopamine neurotransmission is *not* always required for opiate reinforcement (Pettit et al. 1984; Amalric and Koob 1985; Bechara and van der Kooy 1992; Olmstead et al. 1998; Hnasko et al. 2005). Moreover, evidence suggests that within the VTA itself, opiates are capable of producing both dopamine-dependent and dopamine-independent positive reinforcement (Nader and van der Kooy 1997; Laviolette et al. 2004). What biological mechanisms are responsible for this pattern of results? A model capable of organizing these data into a predictive framework would prove very valuable, indeed.

THE NONDEPRIVED/DEPRIVED HYPOTHESIS OF OPIATE MOTIVATION

One attempt to explain the interrelationship between dopamine-dependent and dopamine-independent reinforcement highlighted the importance of the brainstem tegmental pedunculo-pontine nucleus (TPP). It was found that excitotoxic lesions of this area of the brainstem (located near the caudal termination of the medial forebrain bundle, a tract of fibers critical for electrical brain stimulation) (Swanson 1982) could block opiate reinforcement in situations in which disruptions of dopaminergic neurotransmission were ineffective (Bechara and van der Kooy 1992; Olmstead et al. 1998). This model proposed that the split between TPP- and dopamine-based reinforcement could be traced to differences in drug exposure (Bechara and van der Kooy 1992; Nader et al. 1994; Dockstader et al. 2001). More specifically, TPP lesions (but not disruptions of dopamine neurotransmission) only blocked morphine reinforcement in previously opiate-naïve (nondeprived) subjects. Conversely, disruptions of dopamine neurotransmission (but not TPP lesions) were effective in curtailing morphine reinforcement only in subjects that had received

enough drug exposure to render them opiate deprived and in a state of withdrawal (as ascertained by conditioned place aversions and somatic withdrawal signs) (Fig. 1).

However, this change is not permanent. Over time, the “active” motivational system shifts back to the TPP (Nader et al. 1994), indicating that it is not the amount of drug exposure that is important per se but, rather, the relative motivational state (deprived vs. nondeprived) of the organism. Additionally, the motivational effects of food, nicotine, and ethanol also appear to fit this nondeprived/deprived model, although for ethanol the substrates are reversed (dopamine is important in the naive state and the TPP in the dependent and withdrawn state) (Bechara and van der Kooy 1992; Nader and van der Kooy 1994; Laviolette et al. 2002; Laviolette and van der Kooy 2003; Ting-A-Kee et al. 2009).

Direct infusions of morphine into the VTA, in either nondeprived or opiate-deprived sub-

jects, further suggested that this double dissociation between two motivational states (nondeprived and deprived) and two output reinforcement pathways (TPP and dopamine dependent) could be localized to the VTA itself (Nader and van der Kooy 1997). Such a result is partially in accord with previous data positing the VTA as the likely home of a dopamine-dependent motivational signal. However, the obvious question remains: How do opioids produce TPP-dependent *and* dopamine-dependent reinforcement within the VTA? Any potential explanation must take into consideration the anatomy of the VTA.

VTA ANATOMY

The VTA has long been implicated in the study of drug addiction (Wise and Rompre 1989; Kalivas 1993). Dopamine cells make up one of the two predominant cell types of the VTA and are

Figure 1. The nondeprived/deprived hypothesis. (A) A schematic diagram of a rat brain illustrating the basic tenets of the nondeprived/deprived hypothesis of opiate motivation. According to the model, in nondeprived (previously opiate-naïve) subjects, the reinforcing effects of opiates are mediated by a descending projection from the midbrain ventral tegmental area (VTA) to the brainstem tegmental pedunculopontine nucleus (TPP). Conversely, in opiate-deprived (dependent and withdrawn) subjects, the reinforcing effects of opiates are mediated by an ascending dopaminergic projection from the VTA to the nucleus accumbens (NAc). (B) Diagrammatic representation of the basic brain circuitry proposed to be involved in the nondeprived/deprived model.

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more abundant than their γ -amino butyric acid (GABA) counterparts (Swanson 1982; Gysling and Wang 1983; Johnson and North 1992; Kalivas 1993; Ford et al. 1995). More recent reports also suggest the existence of a smaller subpopulation of VTA glutamate cells (Ungless et al. 2004; Yamaguchi et al. 2007; Omelchenko and Sesack 2009; Dobi et al. 2010).

Regulation of the VTA, and of dopamine and GABA neurons in particular, is complex, involving synaptic connections from multiple areas of the brain (Kalivas 1993; Omelchenko et al. 2009; Omelchenko and Sesack 2010). VTA dopamine cells are subject to modulation by a host of local neurotransmitters and various extrinsic efferents (for review, see Kalivas 1993) and make extensive projections to the frontal cortex and various limbic areas (Swanson 1982). Most relevant for our discussion is the large dopaminergic projection to the ventral striatum and the nucleus accumbens (NAc) in particular (Swanson 1982; Ford et al. 2006). It has been suggested that VTA dopamine neurons are not uniform in nature, both in terms of their varying input and output fields (Kalivas 1993; Kalivas and Alesdatter 1993; Blaha et al. 1996; Steffensen et al. 1998; Carr and Sesack 2000; Omelchenko and Sesack 2005; Omelchenko and Sesack 2007) and their structural and electrophysiological properties (Lammel et al. 2008; Margolis et al. 2008).

Similar to the dopamine cells, separate populations of VTA GABA cells also exist in terms of their varying input and output fields (Thierry et al. 1979, 1980; Sesack and Pickel 1992; Kalivas 1993; Van Bockstaele and Pickel 1995; Steffensen et al. 1998; Garzon et al. 1999; Carr and Sesack 2000; Omelchenko and Sesack 2005; Xia et al. 2011). It has been suggested that the GABA cells of the VTA are electrically connected via gap junctions, which may play an important role in VTA dopaminergic reward processes (Steffensen et al. 2011). Additionally, some of these GABA cells send descending projections to the brainstem area, including the TPP (Swanson 1982; Semba and Fibiger 1992; Steininger et al. 1992). More recently, it has been proposed that similar to VTA GABA cells, inhibitory GABAergic projections from the rostromedial mesopontine tegmental nucleus (also known

as the “posterior tail” of the VTA) also synapse with both the TPP (Jhou et al. 2009b) and VTA dopamine cells (Jhou et al. 2009a; Balcita-Pedicino et al. 2011).

Various labeling studies suggest that strong intra-VTA regulation of VTA dopamine, GABA, and glutamate cells also exists (Omelchenko and Sesack 2009; Dobi et al. 2010). Glutamate cells appear to provide local excitatory input to all three cell types (Dobi et al. 2010). Similarly, GABA cells have been described as “upstream” of the dopamine cells and are thought to provide local inhibitory tone to them (Gysling and Wang 1983; Johnson and North 1992; Kalivas 1993; Omelchenko and Sesack 2009), in addition to other local glutamate and GABA neurons themselves (Dobi et al. 2010).

Because the majority of μ -opioid receptors in the VTA are localized to the GABA cells (Dilts and Kalivas 1989; Garzon and Pickel 2001), it has been proposed that μ -opioids produce dopamine cell activation via disinhibition. Direct opioid binding to VTA GABA cells produces hyperpolarization of these neurons (Gysling and Wang 1983; Johnson and North 1992) and, in turn, releases the dopamine cells from their tonic inhibition, allowing for the transmission of a dopamine-dependent reinforcement signal. This proposal is in accord with various dopamine theories of motivation (Wise et al. 1978; Robinson and Berridge 1993; Wise 1996).

However, μ -opioid receptors also are found presynaptically in the VTA, on GABAergic terminals to both VTA GABA and (to a lesser extent) dopamine cell dendrites (Sesack and Pickel 1995; Garzon and Pickel 2001; Svingos et al. 2001; Xia et al. 2011). This raises the possibility that in lieu of (or in addition to) a direct inhibitory action on GABA cells, μ -opioid receptor agonists may disrupt the release of GABA onto either GABA or dopamine neurons. Because GABA typically produces cellular inhibition (Kaila 1994), such a decrease in GABA release onto VTA GABA receptors will usually result in a net excitatory or depolarizing effect on the GABA neurons themselves—although the actual outcome will depend on the specific signaling properties of the GABA receptors themselves.

VTA GABA RECEPTORS

GABA receptors are found distributed across both VTA GABA and dopamine cell populations and, consequently, have been proposed to play a crucial role in dopaminergic cell signaling and motivational processes in general (McBride et al. 1999; Laviolette and van der Kooy 2001; Macey et al. 2001; Walker and Ettenberg 2005; Madhavan et al. 2010). For example, *both* GABA_A receptor agonists and antagonists have been reported to produce positive reinforcing properties when microinjected into the VTA (Ikemoto et al. 1997, 1998; Laviolette and van der Kooy 2001). Although regional specificity—ultimately leading to increased dopamine release—has been proposed to explain this apparent paradox (Ikemoto et al. 1997, 1998), there exists an intriguing alternative. Laviolette and colleagues showed that although both the GABA_A receptor agonist muscimol and the GABA_A receptor antagonist bicuculline produced rewarding effects, they did so via *different* mechanisms (Laviolette and van der Kooy 2001; Laviolette et al. 2004). In previously drug-naïve rats, muscimol's rewarding properties were mediated by dopamine neurotransmission and bicuculline's rewarding properties, by the TPP. Even more surprisingly, these outputs were switched in opiate-dependent and withdrawn rats: now, muscimol produced reinforcement via the TPP and bicuculline, via dopamine. This pattern of results suggested a more complex picture of VTA GABA_A receptor functionality and, perhaps more importantly, suggested that VTA GABA_A receptors may be close to, or even at, the site where the switch between naïve and drug-dependent motivation occurs.

GABA_A receptors are part of a family of ligand-gated ion channels and function primarily as chloride ion channels, although they also possess a significant permeability for bicarbonate ions and other weak acids (Kaila 1994). Each receptor is made up of five subunits (labeled α , β , γ , δ , and ρ), which are, in turn, composed of four transmembrane domains (Kaila 1994; Johnston 1996). The subunits themselves come in many distinct varieties and, in theory, can produce an extremely large

number of subunit combinations, although far fewer appear to exist in nature (Hevers and Ludens 1998). The various combinations of subunits produce differing functional properties (e.g., differences in desensitization) and are often found with a certain degree of regional specificity (Kaila 1994).

In the VTA, GABA_A receptors are found primarily on GABA neurons as indicated by autoradiographic localization (Churchill et al. 1992; Kalivas 1993) or by selective inhibition of non-dopamine (presumably GABAergic) cells by GABA_A receptor-specific drugs (O'Brien and White 1987). On the other hand, GABA_B receptors—which are metabotropic, G-protein-coupled receptors that activate potassium ion channels and/or inhibit calcium channels—mediate slow, prolonged inhibition and are thought to lie primarily on VTA dopamine cells as suggested by antibody staining (Chelib and Johnson 1999; Wirtshafter and Sheppard 2001) and the specific actions of GABA_B receptor agonists on dopamine cells (Olpe et al. 1977; Kalivas et al. 1990; Laviolette and van der Kooy 2001). Figure 2 summarizes the various VTA cell and receptor subtype interrelationships.

GABA_A RECEPTORS POSSESS INHIBITORY AND EXCITATORY SIGNALING PROPERTIES

GABA is the primary inhibitory neurotransmitter in the mammalian brain (Kaila 1994). Activation of ionotropic GABA_A receptors typically results in an inward influx of chloride ions, thereby hyperpolarizing the cell (Kaila 1994; Johnston 1996). This is termed “classical” or “conventional” GABAergic inhibition. However, under certain circumstances, GABA_A receptors are capable of mediating an alternative excitatory/depolarizing response—for example, an efflux of chloride ions (Kaila 1994; Stein and Nicoll 2003). A growing literature has focused on this less well-characterized ability of GABA_A receptors.

In studies of the neonatal hippocampus, activation of GABA_A receptors results in cellular excitation that has been proposed to mediate a trophic signal (Cherubini et al. 1991; Ben-Ari 2002). GABA appears to have excitatory actions

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Figure 2. The anatomy of the ventral tegmental area (VTA). The VTA is composed primarily of dopamine (DA) and GABA neurons, the latter of which are found upstream of the dopamine cells and are thought to provide tonic inhibition of them. GABA_A receptors are localized primarily to GABA cells, and GABA_B receptors to dopamine cells. Both cell types receive projections from areas outside of the VTA, including GABA synapses containing presynaptic μ -opioid receptors. VTA GABA cells also possess μ -opioid receptors. Functionally, the dopamine cells project to the nucleus accumbens and comprise the mesolimbic dopaminergic system, thought to be crucial for the reinforcing properties of opiates in the deprived motivational state. Descending GABA projections to the brainstem tegmental pedunculopontine nucleus (TPP) are thought to be crucial for the non-dopaminergic reinforcing properties of opioids in the nondeprived motivational state.

in many areas of the developing brain; indeed, in vertebrates at least, a developmental shift in GABA_A receptor signaling (from excitatory/depolarizing to inhibitory/hyperpolarizing) appears to be the rule, not the exception (Cherubini et al. 1990; Kaila 1994; Ben-Ari 2002; McCarthy et al. 2002). In addition, the excitatory actions of GABA_A receptors have been proposed as a mechanism for mediating neuropathic pain in the spinal cord (Coull et al. 2003), epileptic seizures in the hippocampus (Rivera et al. 2002), and the ability of the rat suprachiasmatic nucleus to oscillate between daytime excitation and nighttime inhibition (Wagner et al. 1997).

MECHANISMS UNDERLYING THE GABA_A RECEPTOR SIGNALING SWITCH

GABA_A receptors mediate inhibitory chloride ion influxes in the adult brain, where the intra-

cellular chloride concentration is lower than that found extracellularly (Johnston 1996). However, in cases in which the reverse is true (e.g., in the developing brain where the intracellular chloride concentration is higher), GABA activation of GABA_A receptors will result in chloride following its concentration gradient and exiting the cell—an excitatory response (Kaila 1994; Ben-Ari 2002). Consequently, anything that disrupts or alters the relative chloride ion concentration gradient of GABA_A receptor-containing neurons can theoretically provide a mechanism for the excitatory effects of GABA_A receptors.

One idea concerns changes in the activities of various chloride ion cotransporters (Thompson et al. 1988; Kaila 1994; Kahle et al. 2008). For example, the NKCC1 transporter is responsible for the movement of chloride ions into the cell (Ben-Ari 2002; Kahle et al. 2008), and levels of this transporter are reduced

throughout development. This coincides with the developmental change observed in GABA_A receptor signaling, from depolarizing to hyperpolarizing (Liu et al. 2006; Kahle et al. 2008). Conversely, down-regulation (or decreased activities) of chloride transporters that remove intracellular chloride—such as KCC transporters—would have the opposite effect, allowing chloride to accumulate intracellularly (Thompson et al. 1988). Research suggests that these KCC transporters are increased during development (Rivera et al. 1999; Hubner et al. 2001). This increase would improve the clearance of chloride and facilitate the loss of GABA_A receptor excitability in developing neurons.

Another potential mechanism of GABA_A receptor excitability concerns the ability of other ions to traverse GABA_A receptors. Although known primarily as chloride channels, GABA_A receptors also allow the passage of other select molecules (Kaila 1994). Chief among these are bicarbonate ions, which possess about one-fifth the permeability of chloride ions and are found in physiologically relevant concentrations within the cell (Kaila 1994). Bicarbonate concentrations are usually greater inside of the cell than outside, and consequently bicarbonate flows out of the cell upon opening of the GABA_A receptor channel, an action that produces cellular excitation. Although chloride is generally more abundant and possesses a higher permeability than bicarbonate ions, should the chloride ion concentration gradient break down—perhaps, because of an excessive influx of chloride in response to multiple receptor activations—the bicarbonate ion electrochemical gradient has been proposed to “take over” and drive the overall response of GABA_A receptor activation (Kaila et al. 1993; Staley et al. 1995). Indeed, high-frequency stimulation of rat hippocampus pyramidal neurons produces precisely such an excitatory GABA_A receptor effect (Kaila et al. 1997; Staley and Proctor 1999).

In line with this idea, some investigators have proposed manipulating the concentration of bicarbonate ions as a means of controlling the response properties of GABA_A receptors—that is, artificially “switching” them to mediate

excitation as opposed to inhibition. Bicarbonate ions are generated by the enzyme carbonic anhydrase, which catalyzes its synthesis from water and carbon dioxide (Ridderstrale and Wistrand 2000; Kida et al. 2006). By controlling the activity of this enzyme, it was proposed that one could control the intracellular concentration of bicarbonate and thereby influence the response properties of GABA_A receptors. According to this idea, inhibition of the enzyme should prevent bicarbonate buildup and, therefore, prevent any bicarbonate-mediated depolarizing response. Conversely, activation of the enzyme should have the opposite effect, increasing the intracellular bicarbonate concentration and encouraging GABA_A receptor-mediated depolarization. To this end, inhibition of the carbonic anhydrase enzyme with the drug acetazolamide was found to reduce neuropathic allodynia caused by GABA_A receptor-mediated depolarization (Asiedu et al. 2010), and activation of the carbonic anhydrase enzyme was found to facilitate rodent spatial memory in a Morris water maze test (Sun and Alkon 2001, 2002). Figure 3 shows the proposed GABA_A receptor-relevant ion flows.

Changes in GABA_A receptor subunits also have been proposed as a mechanism to explain changes in GABA_A receptor responses. Research suggests that at least some GABA_A receptor subunits are altered during the course of development, leaving open the possibility that they are involved in the switch in GABA_A receptor signaling (Fritschy et al. 1994). Additionally, the existence of GABA-gated cation channels has been shown in the nematode *Caenorhabditis elegans* (Beg and Jorgensen 2003), suggesting that excitation-specific GABA receptors exist (although whether such receptors are found in vertebrates remains unknown).

In summary, it is clear that GABA_A receptors are capable of producing both inhibitory and excitatory modes of signaling. Several mechanisms have been proposed to explain how this is accomplished. The differing signaling properties of GABA_A receptors underlie a large number of biological processes and, as discussed below, also appear to play a central role in opiate motivation.

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Figure 3. Ion movements in VTA GABA_A receptors. Activation of VTA GABA_A receptors located on GABA neurons allows for chloride (Cl⁻) and bicarbonate (HCO₃⁻) ions to flow down their electrochemical gradients. In the adult, chloride is usually found in greater abundance extracellularly, and, therefore, GABA_A receptor activation produces an influx of chloride, hyperpolarizing the cell. Conversely, bicarbonate is more concentrated intracellularly and therefore flows out of the cell, an excitatory response. Bicarbonate production (from carbon dioxide and water) is catalyzed by the carbonic anhydrase enzyme and can be inhibited by the drug acetazolamide, thereby negating any bicarbonate-mediated excitation. The relative intracellular chloride ion concentrations are maintained by the NKCC and KCC transporters, which move chloride into and out of the cell, respectively. In adults, levels of the NKCC channel are relatively low. Consequently, blockade of KCC transporter activity (with furosemide) helps to decrease any inward-directed chloride ion flow, facilitating GABA_A receptor excitation.

VTA GABA_A RECEPTORS FORM A MOTIVATIONAL SWITCHING MECHANISM FOR OPIATES

According to the nondeprived/deprived hypothesis, the prior drug history of the animal (previously drug naive or drug dependent and in a state of withdrawal) determines the substrate mediating opiate reinforcement. The “switch” version of this model proposes that changes in VTA GABA_A receptors are the crucial intermediary linking (1) motivational state, and (2) output reinforcement pathway.

Both TPP- and dopamine-dependent reinforcing effects have been observed with intra-VTA opiate infusions (Nader and van der Kooy 1997; Laviolette and van der Kooy 2004). Therefore, VTA GABA cells were proposed to act as a natural “switch” capable of shuttling opiate reinforcement between these two pathways, because these neurons possess anatomical connections with both VTA dopamine cells and the

TPP (although not necessarily by the same GABA cell) (Swanson 1982; Semba and Fibiger 1992; Steininger et al. 1992; Kalivas 1993; Laviolette et al. 2004; Omelchenko and Sesack 2009). However, the exact mechanics of this process remained problematic. How could a single GABA cell (or group of cells) shift its signaling properties to mediate either TPP- or dopamine-dependent reinforcement?

One elegant solution lay in the dual signaling properties of the VTA GABA_A receptors themselves. Recall that GABA_A receptors are mainly localized to VTA GABA neurons (Churchill et al. 1992; Kalivas 1993) and are capable of producing two distinctive modes of signaling (Kaila 1994; Stein and Nicoll 2003). These excitatory and inhibitory modes of action have previously been proposed to mediate several biological processes (Cherubini et al. 1990, 1991; Wagner et al. 1997; Rivera et al. 1999; Auger et al. 2001; McCarthy et al. 2002; Coull et al.

2003; Leitch et al. 2005). Similarly, it was proposed that the TPP- and dopamine-dependent reinforcement pathways observed by investigators might have their biological bases rooted in the biphasic actions of VTA GABA_A receptors (Laviolette et al. 2004; Vargas-Perez et al. 2009). Such a model would require that opiates exert their actions (at least initially in drug-naïve subjects) on the μ -opioid receptors found presynaptically in the VTA, which regulate GABA release onto GABA_A receptors (Fig. 2) (Sesack and Pickel 1995; Garzon and Pickel 2001; Svingos et al. 2001; Xia et al. 2011).

Electrophysiological, molecular, and behavioral evidence supports the idea that a switch from an inhibitory to an excitatory VTA GABA_A receptor does, in fact, occur in animals that have changed from a previously drug-naïve motivational state, to an opiate-dependent and withdrawn state (Laviolette et al. 2004; Vargas-Perez et al. 2009). In previously drug-naïve rats, the GABA_A receptor agonist muscimol produced inhibition of GABA neurons via their direct actions on GABA_A receptors (Laviolette et al. 2004). However, once the rats were opiate dependent and in withdrawal, muscimol now produced excitation in a significant subset of these same GABA cells, suggesting that at least some of the GABA_A receptors had switched to mediate excitation (Laviolette et al. 2004; Vargas-Perez et al. 2009). Similarly, CREB phosphorylation (a marker of excitatory GABA_A receptor activity) of VTA GABA cells was increased in opiate-dependent and withdrawn rats as compared with their previously drug-naïve counterparts (Laviolette et al. 2004).

Furthermore, experiments using opposing artificial manipulations of VTA GABA_A receptors produced opposite effects on opiate motivation. Acetazolamide—which inhibits the carbonic anhydrase enzyme, thereby preventing the generation of intracellular bicarbonate and therefore, any bicarbonate-mediated excitation (Fig. 3)—had no effect in previously drug-naïve animals (Laviolette et al. 2004; data not shown). However, in opiate-dependent and withdrawn animals, acetazolamide prevented the block of both intra-VTA and systemic morphine place preferences that is normally ob-

served in subjects also pre-treated with the broad-spectrum dopamine receptor antagonist α -flupenthixol. Conversely, furosemide—which blocks the KCC chloride channel that removes intracellular chloride, thereby facilitating bicarbonate-mediated excitation (Fig. 3)—had no effect in opiate-dependent and withdrawn animals. However, in previously drug-naïve animals that had received lidocaine inactivation of the TPP (which normally blocks morphine place preferences), morphine place preferences were instead revealed. This furosemide effect was reversed by coadministration with acetazolamide, suggesting that the latter's actions on the carbonic anhydrase enzyme (and, therefore, on the generation of bicarbonate ions) may be a final common downstream pathway for the depolarizing actions of VTA GABA_A receptors.

These data argue convincingly in favor of the idea that during the change to an opiate-deprived animal, VTA GABA_A receptors undergo a parallel switch in signaling properties. This VTA-based switch is crucial to determining whether opiate reinforcement is TPP or dopamine dependent.

BRAIN-DERIVED NEUROTROPHIC FACTOR IS CRITICAL FOR THE VTA GABA_A RECEPTOR SWITCHING MECHANISM

Although the above experiments suggest that opiate reinforcement is, indeed, controlled by a VTA-switching mechanism, it remains to be determined what natural mediator(s) is responsible for instigating this switch naturally when animals undergo a change from a previously drug-naïve to an opiate-dependent and withdrawn motivational state. One possibility is the neurotrophic factor brain-derived neurotrophic factor (BDNF). There is a wealth of data suggesting that BDNF plays an important role in various aspects of drug addiction (Hall et al. 2003; Lu et al. 2004; Berglind et al. 2007; Lobo et al. 2010). More relevant for this discussion are reports suggesting that BDNF is capable of producing a change in the signaling properties of GABA_A receptors (Rivera et al. 2002; Coull et al. 2005). Indeed, BDNF has been

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linked to decreases in the activity of neuronal KCC transporters (Rivera et al. 2002; Wake et al. 2007). Perhaps not so coincidentally, during the change from a previously drug-naïve to an opiate-dependent and withdrawn animal, levels of VTA BDNF also are increased (Vargas-Perez et al. 2009). A BDNF-induced decrease in the activity of the KCC transporter on VTA GABA cells should result in the intracellular accumulation of chloride ions (similar to the proposed effect of furosemide), which, in turn, might allow for another ion flow—such as bicarbonate extrusion—to dominate and produce a depolarizing response (Staley et al. 1995). Such a mechanism might be sufficient to explain the observed switch in VTA GABA_A receptor signaling during the change from a previously opiate-naïve, to an opiate-dependent and withdrawn motivational state (Laviolette et al. 2004; Vargas-Perez et al. 2009). Further support for this idea comes from the fact that intra-VTA infusion of siRNA—targeted to the high-affinity BDNF trkB receptor—prevents the switch to a dopamine-dependent reinforcement system even in opiate-dependent and withdrawn animals (data not shown). It is also possible that other potential actions of BDNF (e.g., on GABA_A receptor subunit composition, cell surface expression, and cortical excitability) (Rutherford et al. 1997; Thompson et al. 1998; Brunig et al. 2001) may play a role in this process. Overall, the evidence supports the idea that BDNF—released naturally in response to opiate intake—mediates the switch in VTA GABA_A receptor signaling and, therefore, the switch in opiate reinforcement mechanisms.

AN INTEGRATED MODEL OF OPIATE MOTIVATION IN THE VTA

According to the model, opiates exert their functional effects by binding to upstream μ -opioid receptors located on presynaptic GABA-releasing terminals in the VTA (and not those μ -opioid receptors located on the GABA cells themselves) (Garzon et al. 1999; Svingos et al. 2001; Omelchenko and Sesack 2009; Xia et al. 2011). This results in a decrease in tonic GABA release onto VTA GABA_A receptors, which are located

on GABA perikarya. This decreased activation of GABA_A receptors has different consequences depending on the motivational state of the organism.

In nondeprived, previously drug-naïve animals, activation of GABA_A receptors inhibits VTA GABA cells (including those GABA cells that provide local inhibition to VTA dopamine cells). Opiate binding to presynaptic μ -opioid receptors decreases this inhibitory response (functionally similar to the direct antagonist actions of bicuculline on VTA GABA_A receptors) and, consequently, depolarizes the GABA cells. This results in (1) continued inhibition of the dopamine cells, and (2) the initiation of a reinforcement signal from VTA GABA cells to the TPP. Thus, opiate reinforcement in drug-naïve animals is TPP dependent (Fig. 4).

Conversely, in opiate-deprived subjects where activation of VTA GABA_A receptors excites GABA cells, opiate administration would have the exact opposite effect. Opiate binding to presynaptic μ -receptors again reduces GABA release onto GABA_A receptors. This loss of an excitatory input causes VTA GABA cells to hyperpolarize (this same effect would be observed if opiates now bound to μ -opioid receptors located directly on the VTA GABA cells themselves). Consequently, (1) no reinforcement signal is sent to the TPP, and (2) the inhibition of VTA GABA cells disinhibits the dopamine cells, leading to the production of a dopamine-dependent reinforcement signal (Fig. 4). The simplest version of this model assumes that the VTA GABA cells important for the switching mechanism project to both the TPP and local dopamine cells, although it is possible that separate GABA cell populations exist instead.

SUMMARY

The nondeprived/deprived “switch” hypothesis suggests that GABA_A receptors on VTA GABA neurons form a switching mechanism that controls the substrates underlying opiate motivation. In lieu of opiates acting upon separate sites of action (to produce TPP- and dopamine-dependent reinforcement), the opioid site of

Figure 4. An integrated model of opiate reinforcement in the ventral tegmental area (VTA). The activation of VTA GABA_A receptors in nondeprived animals (*left* side) produces hyperpolarization of VTA GABA cells. Binding of opiates to presynaptic μ -receptors reduces their activation; consequently, VTA GABA cells become depolarized because of the loss of an inhibitory input. In turn, the GABA cells maintain their inhibition of the VTA dopamine cells and send a descending opiate reinforcement signal to the tegmental pedunclopontine nucleus (TPP). Conversely, in opiate-dependent and withdrawn (deprived) animals (*right* side), activation of VTA GABA_A receptors produces depolarization of VTA GABA cells. Opiate binding to presynaptic μ -receptors still results in a reduction of VTA GABA_A receptor activation, leading to VTA GABA cell hyperpolarization (due to the loss of an excitatory input). VTA dopamine cells now become disinhibited and increase their firing, sending a dopamine-dependent reinforcement signal to the nucleus accumbens (NAc).

action is proposed to remain constant, and a common downstream effector—VTA GABA_A receptors—instead change their functional properties. Manipulation of this functionality—by controlling whether VTA GABA_A receptor activation produces inhibition or excitation—controls the active opiate reinforcement output system (TPP or dopamine dependent), irrespective of the animal's previous drug history. This change is temporary and may be naturally mediated by the actions of BDNF in the VTA. The nondeprived/deprived “switch” model thus provides an experimentally tested, predictive framework for organizing reports of both dopamine-dependent and dopamine-independent opioid reinforcement. This result is an important step in our continued attempt to explore the mechanisms underlying motivation

and suggests that an emphasis on research in drug-deprived subjects may be a worthwhile endeavor.

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