

# Endocannabinoids Shape Accumbal Encoding of Cue-Motivated Behavior via CB1 Receptor Activation in the Ventral Tegmentum

Erik B. Oleson,<sup>1</sup> Michael V. Beckert,<sup>1</sup> Joshua T. Morra,<sup>3</sup> Carien S. Lansink,<sup>1</sup> Roger Cachope,<sup>1</sup> Rehab A. Abdullah,<sup>4</sup> Amy L. Loriaux,<sup>5</sup> Dustin Schetters,<sup>6</sup> Tommy Pattij,<sup>6</sup> Mitchell F. Roitman,<sup>5</sup> Aron H. Lichtman,<sup>4</sup> and Joseph F. Cheer<sup>1,2,\*</sup>

<sup>1</sup>Department of Anatomy and Neurobiology

<sup>2</sup>Department of Psychiatry

University of Maryland School of Medicine, Baltimore, MD 21201, USA

<sup>3</sup>Center for Neuropharmacology and Neuroscience, Albany Medical College, Albany, NY 12208, USA

<sup>4</sup>Department of Pharmacology and Toxicology, Medical College of Virginia Campus, Virginia Commonwealth University, Richmond, VA 23298, USA

<sup>5</sup>Department of Psychology, University of Illinois at Chicago, Chicago, IL 60607, USA

<sup>6</sup>Department of Anatomy and Neurosciences, Neuroscience Campus Amsterdam, VU University Medical Center, 1081 HV Amsterdam, The Netherlands

\*Correspondence: [jchee001@umaryland.edu](mailto:jchee001@umaryland.edu)

DOI 10.1016/j.neuron.2011.11.018

## SUMMARY

Transient increases in nucleus accumbens (NAc) dopamine concentration are observed when animals are presented with motivationally salient stimuli and are theorized to energize reward seeking. They arise from high-frequency firing of dopamine neurons in the ventral tegmental area (VTA), which also results in the release of endocannabinoids from dopamine cell bodies. In this context, endocannabinoids are thought to regulate reward seeking by modulating dopamine signaling, although a direct link has never been demonstrated. To test this, we pharmacologically manipulated endocannabinoid neurotransmission in the VTA while measuring transient changes in dopamine concentration in the NAc during reward seeking. Disrupting endocannabinoid signaling dramatically reduced, whereas augmenting levels of the endocannabinoid 2-arachidonoylglycerol (2AG) increased, cue-evoked dopamine concentrations and reward seeking. These data suggest that 2AG in the VTA regulates reward seeking by sculpting ethologically relevant patterns of dopamine release during reward-directed behavior.

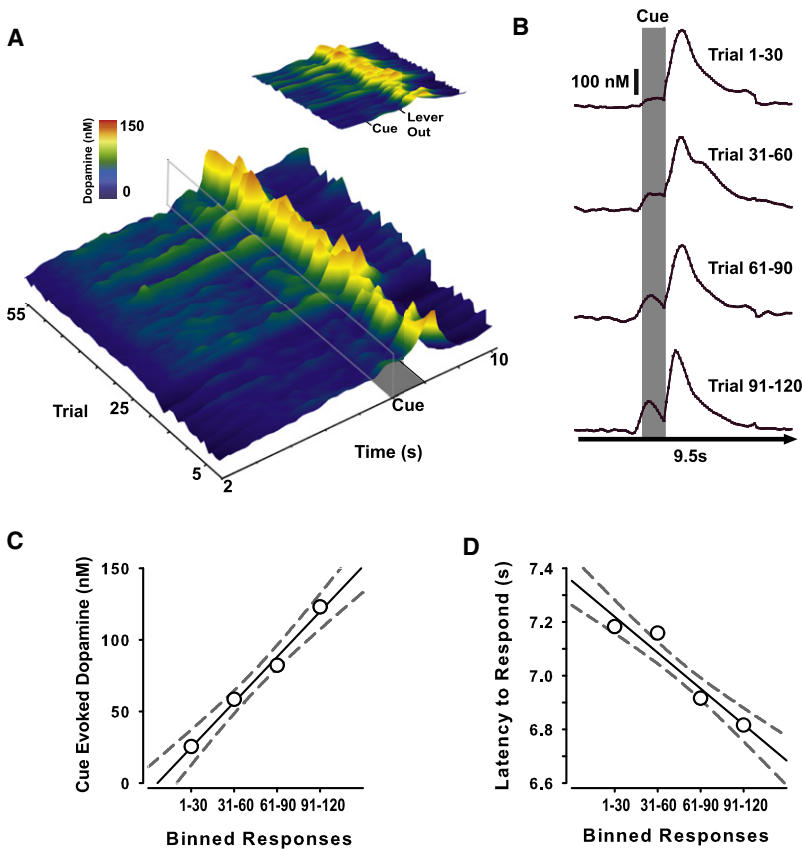
## INTRODUCTION

The neural mechanisms responsible for the pursuit of rewards in the environment are essential for the survival of the organism (Nesse and Berridge, 1997; Schultz et al., 1997). Environmental stimuli that predict the availability of reward develop incentive-motivational properties that energize the seeking of future rewards (Bindra, 1968). The NAc is a neural substrate that is critically involved in integrating interoceptive and environmental

information with emotional information to initiate reward seeking (Kelley, 1999; Mogenson et al., 1980). When reward seeking is maintained in a controlled experimental setting in which environmental stimuli predict reward availability, transient dopamine surges in the NAc begin to occur in response to the predictive stimuli (i.e., conditioned cues) following the attribution of incentive salience (Berridge and Robinson, 1998; Flagel et al., 2011). These transient increases in dopamine have been detected in the NAc when animals are presented with cues predicting various rewards—including drugs of abuse (Phillips et al., 2003), food (Roitman et al., 2004), and brain stimulation reward (Cheer et al., 2007a)—and are required to promote reward-directed behavior (Nicola, 2010).

The brain endocannabinoid system, formed by metabotropic cannabinoid receptors (CB1 and CB2) and their endogenous ligands (e.g., anandamide and 2AG), is important for the regulation of dopamine signaling during reinforcement processing (Lupica and Riegel, 2005; Solinas et al., 2008). When dopamine neurons in the VTA exhibit brief high-frequency firing episodes they release endocannabinoids that act as retrograde messengers by binding to pre-synaptic CB1 receptors, thereby indirectly modulating the excitability of dopamine neurons by reducing presynaptic neurotransmitter release (Mellis et al., 2004). Rather than being released through a vesicular mechanism, endocannabinoids are distinct from other neurotransmitters in that they are formed and released “on demand” during specific neural events (Freund et al., 2003). It is likely, therefore, that endocannabinoids regulate dopamine signaling during reward seeking exclusively in situations in which dopamine neurons fire at high frequencies—like when animals are presented with environmental cues predicting reward (Schultz et al., 1997).

To investigate whether endocannabinoids modulate the neural mechanisms of reward seeking, we measured changes in the concentration of cue-evoked dopamine transients in the NAc shell while pharmacologically altering endocannabinoid signaling during operant behavior. A pharmacological approach



**Figure 1. Reward Seeking and Cue-Evoked Dopamine Release Events Are Strengthened across Trials**

(A) Pavlovian associations sculpt patterns of transient dopamine release in response to a predictive cue during reward seeking. A representative surface-plot shows changes in dopamine concentration (z axis) occurring across trials (y axis) while responding is maintained by brain stimulation reward in an ICSS task. Cue presentation, which is indicated by the gray rectangle, occurred for 1 s prior to lever extension.

(B) Representative traces show the mean cue-evoked dopamine concentration increasing across trials. Each dopamine concentration trace represents the mean of 30 consecutive trials.

(C and D) The conditioned cue begins to strengthen reward seeking as the concentration of cue-evoked dopamine increases across trials. Linear regression analyses show increases in mean dopamine concentration and decreases in response latency across binned responses. Dashed lines represent the 95% confidence region.

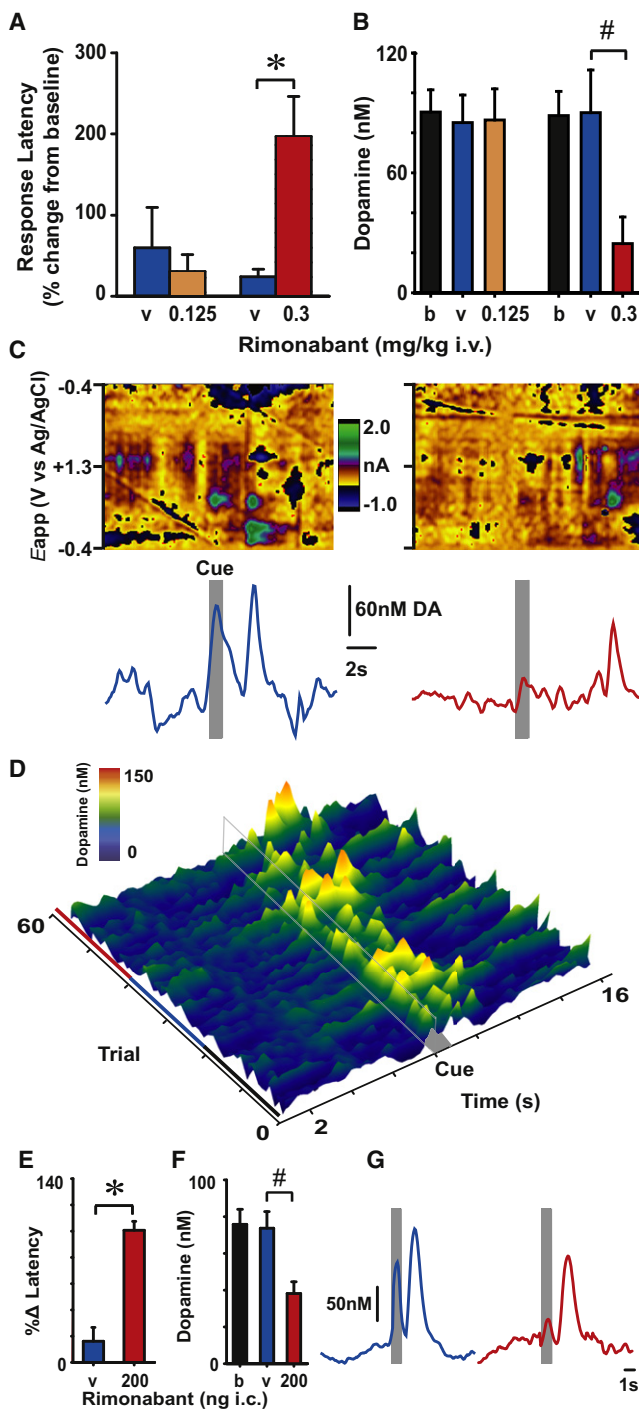
was chosen because we previously demonstrated that blocking CB1 receptors using rimonabant (a CB1 receptor antagonist) reduced drug-induced transient dopamine release into the NAC (Cheer et al., 2007b). Operant behavior was maintained by either brain stimulation reward or food reinforcement while an environmental cue signaled the availability of reward. We found that disrupting endocannabinoid signaling uniformly decreased the concentration of cue-evoked dopamine transients and reward seeking. These findings prompted us to investigate whether increasing endocannabinoid levels would facilitate reward seeking, and if so, which endocannabinoid is responsible. Using recently developed pharmacological tools designed to manipulate specific components of the endocannabinoid system, we found that augmenting 2AG, but not anandamide, levels by disrupting metabolic enzyme activity increased dopamine signaling during reward seeking—suggesting that 2AG sculpts ethologically relevant patterns of dopamine release during reward-directed behavior.

## RESULTS

### Transient Dopamine Concentrations Time Locked to Cue Presentation Develop across Trials

Dopamine was measured in the NAc shell using fast-scan cyclic voltammetry (FSCV) while responding was maintained in a previously described intra-cranial self-stimulation (ICSS) task (Cheer et al., 2007a). As in our previous report (Cheer et al., 2007a),

a compound cue predicted reward availability. This occurred across multiple sensory modalities; specifically, a house light turned off, an ongoing tone ceased, and then 1 s later a white stimulus light mounted above the lever was presented simultaneously with lever extension. A 10 s timeout followed each lever response. Under these conditions, electrically-evoked dopamine release occurred following a lever response and was temporally dissociable from cue-evoked dopamine release events, allowing for changes in the concentration of cue-evoked dopamine to be measured across trials. In agreement with previous studies (Day et al., 2007; Owesson-White et al., 2008), the concentration of dopamine occurring in response to the cue during this acquisition session increased across trials (Figures 1A and 1B). While the concentration of cue-evoked dopamine rapidly increased (Figure 1C;  $R^2 = 0.85$ ;  $n = 5$ ), the latency to respond from lever extension (a metric of reward seeking) decreased in a linear fashion (Figure 1D;  $R^2 = 0.80$ ;  $n = 5$ ; mean values: 7.18, 7.16, 6.91, 6.81 s), demonstrating that the strengthening of Pavlovian associations between the cue and unconditioned stimulus is accompanied by increased and cue-related dopamine signaling (Day et al., 2007). Importantly, increased recruitment of endocannabinoids in the VTA should develop in association with an increasing concentration of cue-evoked dopamine release. As dopamine neurons fire in high frequency bursts, voltage gated  $Ca^{2+}$  ion channels open and the resulting  $Ca^{2+}$  influx activates the enzymes responsible for the synthesis of endocannabinoids (Wilson and Nicoll, 2002). Thus, endocannabinoid levels should be highest in the VTA after periods of phasic dopamine neural activity. If endocannabinoids are indeed involved in modulating dopamine signaling during reward seeking, pharmacological disruption of endocannabinoids should decrease cue-evoked dopamine concentrations and cue-motivated responding in unison.



**Figure 2. Disrupting Endocannabinoid Signaling Decreases Cue-Evoked Dopamine Concentrations during Reward Seeking in an ICSS Task**

(A) Response latency (a metric of reward seeking) for brain stimulation reward maintained in the ICSS task. A high (0.3 mg/kg i.v.; red bar) but not low (0.125 mg/kg i.v.; orange bar) dose of rimonabant increased the latency to respond for brain stimulation reward in comparison to vehicle (v, blue bar). (B) Mean dopamine concentration observed during the first second of cue presentation under baseline (b), vehicle (v), and drug conditions. Rimonabant at a high (0.3 mg/kg i.v.; red bar) but not low (0.125 mg/kg i.v.; orange bar) dose

**Disrupting Endocannabinoid Signaling during ICSS Decreases Cue-Evoked Dopamine Concentrations and Reward Seeking**

To assess the effects of disrupting endocannabinoid signaling on cue-evoked dopamine concentrations and reward seeking, we treated rats with the CB1 receptor antagonist rimonabant while responding was maintained by brain stimulation reward in an ICSS task. Following the establishment of stable baseline concentrations of cue-evoked dopamine release, animals were given access to 30 stimulations for each component of the session (i.e., baseline, vehicle, and drug treatment). A high (0.3 mg/kg i.v.; MWU test,  $U = 3$ ,  $p < 0.01$ ;  $n = 15$ ; mean values:  $b = 0.91$ ,  $v = 1.09$ ,  $rimo = 2.45$  s) but not low (0.125 mg/kg i.v.) rimonabant dose increased the latency to respond for brain stimulation reward (Figure 2A) in comparison to vehicle treatment. The increase in response latency was accompanied by a decrease in the concentration of cue-evoked dopamine (Figure 2B;  $F_{(2,44)} = 5.40$ ,  $p < 0.01$ ; 0.3 mg/kg versus vehicle,  $p = 0.02$ ; also see Figure S1A available online for mean dopamine concentration traces). Cue-evoked dopamine concentrations were not affected by the lower rimonabant dose (Figure 2B; 0.125 mg/kg i.v.). Representative color plots and accompanying dopamine concentration traces (Figure 2C) show rimonabant (0.3 mg/kg i.v.) decreasing cue-evoked dopamine events during individual trials, whereas the representative surface plot (Figure 2D) illustrates the effect of rimonabant (0.3 mg/kg i.v.) on dopamine concentrations across trials. We further determined that the decreases in reward seeking and cue-evoked dopamine concentration could not be explained by a drug-induced effect on electrically-evoked dopamine release (Figure S1B), consistent with an absence of CB1 receptors on dopamine terminals (Julian et al., 2003) and could be replicated using the more

decreased the concentration of cue-evoked dopamine in comparison to vehicle.

(C) Representative color plots (top) and dopamine concentration traces (bottom) show the effects of rimonabant on cue-evoked dopamine events in individual trials. Top: Representative color plots topographically depict the voltammetric data with time on the x axis, applied scan potential ( $E_{app}$ ) on the y axis and background-subtracted faradaic current shown on the z-axis in pseudocolor. Dopamine can be identified by an oxidation peak (green) at +0.6 V and a smaller reduction peak (yellow) at -0.2 V. Bottom: Corresponding traces show the concentration of dopamine (nM) detected at the time of cue presentation (gray bar) following vehicle (left; blue trace) and rimonabant (right; red trace) administration.

(D) A representative surface-plot shows changes in dopamine concentration (z axis) across trials (y axis) during baseline (black line), vehicle (blue line), and rimonabant (red line) conditions. Data are centered around lever presentation on the x axis.

(E) Disrupting endocannabinoid signaling within the VTA is sufficient to decrease reward seeking. Intrategmental rimonabant (200 ng i.c.; red bar) significantly increased response latency in comparison to vehicle (v, blue bar). (F) Mean dopamine concentrations observed during first second of cue-presentation under baseline (b), vehicle (v), and drug conditions. Intrategmental rimonabant (200 ng i.c.; red bar) significantly decreased the concentration of cue-evoked dopamine in comparison to vehicle.

(G) Representative dopamine concentration traces from individual trials after vehicle (left; blue trace) and rimonabant (200 ng i.c.; right; red trace) treatment. Traces represent individual data, bars represent mean values, and error bars represent  $\pm$  SEM. A significant difference versus vehicle is indicated by either \* ( $p < 0.01$ ) or # ( $p < 0.05$ ).

selective CB1 receptor antagonist AM251 (Figure S1C). Next we sought to establish if disrupting the VTA endocannabinoid system alone is sufficient to decrease dopamine neurotransmission by infusing rimonabant directly into the VTA during reward seeking maintained in the ICSS task. As was found following systemic treatment, intrategmental rimonabant (200 ng i.c., unilateral) significantly increased the latency to respond for brain stimulation reward (Figure 2E; MWU test,  $U = 0$ ,  $p < 0.01$ ;  $n = 8$ ; mean values:  $b = 0.94$ ,  $v = 1.10$ ,  $rimo = 1.96$  s) and decreased cue-evoked dopamine concentrations (Figure 2F;  $F_{(2,14)} = 7.01$ ,  $p < 0.01$ ; 200 ng versus vehicle,  $p = 0.03$ ; also see Figure S4A for mean dopamine concentration traces). The representative dopamine concentration traces (Figure 2G) show the effect of intrategmental rimonabant on cue-evoked dopamine events in individual trials. Rimonabant-induced decreases in cue-evoked dopamine concentration during reward seeking maintained in the ICSS task can also be observed in audio-visual format (Movie S1). These data demonstrate that the VTA endocannabinoid system modulates dopamine signaling during the pursuit of brain stimulation reward.

#### Disrupting Endocannabinoid Signaling during Food Self-Administration Decreases Cue-Evoked Dopamine Concentrations and Appetitive Food Seeking

To assess whether disrupting endocannabinoid signaling also decreases dopamine transmission during the pursuit of natural reward, we treated animals with rimonabant while responding was maintained in an appetitive food-seeking task (Supplemental Experimental Procedures). Similar to the ICSS task, each lever response resulted in the delivery of food reinforcement and retraction of the lever for 10 s. After each 10 s timeout, a compound cue indicating reward availability was presented simultaneously with lever extension. Rimonabant decreased food seeking, as both a low (0.125 mg/kg i.v.; MWU test,  $U = 4$ ,  $p = 0.03$ ;  $n = 6$ ) and high (0.3 mg/kg i.v.; MWU test,  $U = 0$ ,  $p < 0.01$ ;  $n = 8$ ; mean values:  $b = 1.45$ ,  $v = 1.82$ ,  $rimo = 17.7$  s) dose increased response latency in comparison to vehicle treatment (Figure 3A). Rimonabant was administered prior to 60 responses, before animals reached satiety levels (avg. of 200 reinforced responses). As in the ICSS task, an increase in response latency was accompanied by a decrease in the concentration of cue-evoked dopamine release (Figure 3C;  $F_{(2,14)} = 5.87$ ,  $p < 0.01$ ; 0.3 mg/kg versus vehicle,  $p = 0.04$ ; also see Figure S2A for mean dopamine concentration traces). Rimonabant-induced decreases in cue-evoked dopamine concentration during individual (Figure 3D) and repeated (Figure 3E) trials are illustrated in pseudocolor. Likewise, intrategmental rimonabant-induced increases in response latency (Figure 3F; MWU test,  $u = 0$ ,  $p < 0.01$ ;  $n = 5$ ; mean values:  $b = 1.18$ ,  $v = 1.3$ ,  $rimo = 2.75$  s) were accompanied by a decrease in cue-evoked dopamine concentration (Figure 3G;  $F_{(2,14)} = 9.86$ ,  $p < 0.01$ ; 200 ng versus vehicle,  $p = 0.014$ ; also see Figure S4B for mean dopamine concentration traces). Representative traces showing the effects of vehicle and intrategmental rimonabant (200 ng i.c., unilateral) on cue-evoked dopamine events are illustrated in Figure 3H. Rimonabant-induced decreases in food seeking can also be observed by viewing audio-visual material (Movie S2). Together, these data demonstrate that disrupting

the VTA endocannabinoid system alone is sufficient to decrease natural reward seeking.

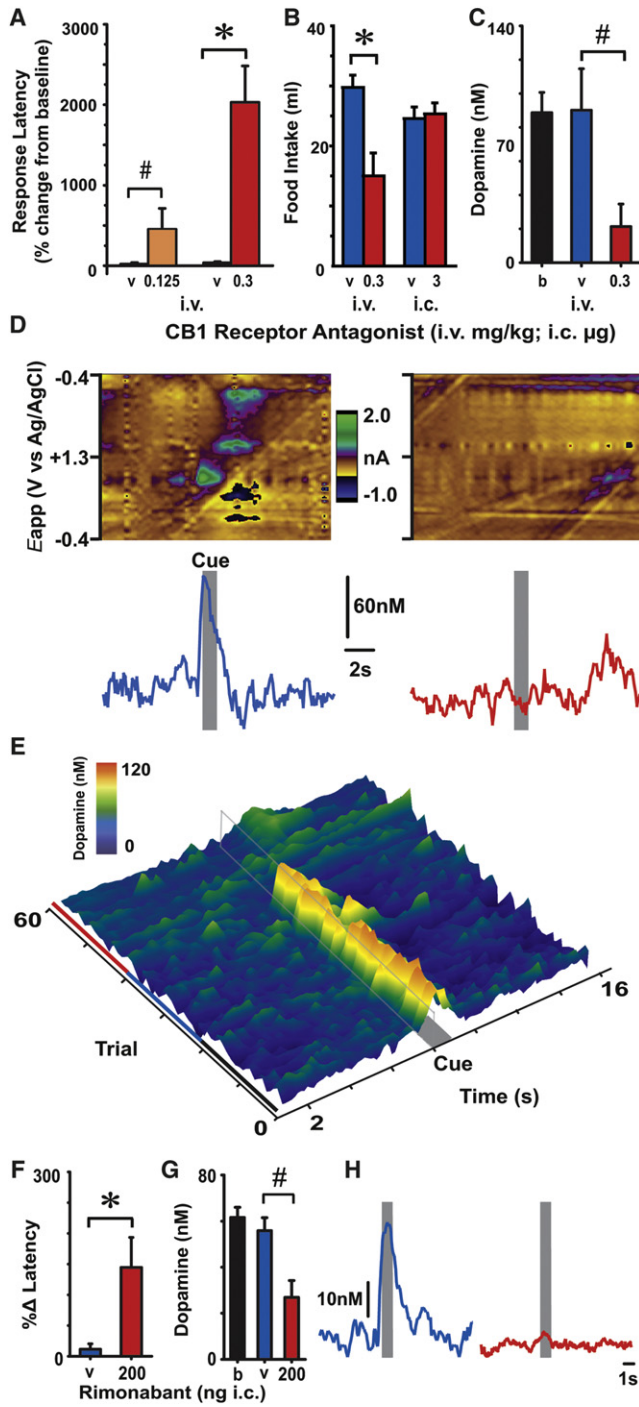
Cannabinoid receptors are abundantly expressed throughout the central and peripheral nervous system, however, and are known to regulate consummatory behavior at a systems level (Gomez et al., 2002; Berry and Mechoulam, 2002). We therefore tested whether rimonabant-induced decreases in food seeking can be explained by a decrease in consummatory behavior rather than a decrease in appetitive food seeking by measuring preferred meal size in an intraoral intake task (Supplemental Experimental Procedures). Appetitive behavior involves a pursuit of reward in the environment and is influenced by the motivational state of the animal (Bindra, 1968; Kelley, 1999), whereas consummatory behavior involves the regulation of intake and is reflected by an animal's preferred meal size (Foltin and Haney, 2007). Intrategmental CB1 receptor antagonists did not produce changes in cumulative intraoral intake (Figure 3B, right;  $t_{(6)} = 0.3$ , n.s.) but significantly decreased intake when administered systemically (Figure 3B, left;  $t_{(6)} = -3.4$ ,  $p < 0.01$ ), suggesting that the VTA endocannabinoid system exclusively regulates appetitive aspects of feeding behavior.

Although the doses of rimonabant used in the present study are comparable to those previously shown to reduce the effects of environmental stimuli on motivated behavior without producing nonspecific effects on locomotor activity (Le Foll and Goldberg, 2004), we wanted to further assess whether our reported decreases in reward seeking resulting from CB1 receptor antagonism might be explained by a disruption in either attentional processing or motor performance by assessing the effects of rimonabant on behavior maintained in the five-choice serial reaction time task. Rimonabant (0.3 mg/kg i.v.) failed to disrupt visuospatial attention, as assessed by accurate choice (Figure S2B) or motor performance, as measured by the latency to respond to visual stimuli (Figure S2C). These data support that the rimonabant-induced decreases presented herein are due to a specific effect on reward seeking rather than nonspecific behavioral effects on attention or operant performance.

#### Interval Timing, Dopamine Release, and Endocannabinoids

In confirmation of our previous report (Cheer et al., 2007a), we observed increases in dopamine concentration preceding cue presentation (Figures 1B, 2C, 3D, and 3H). These data support the theory that dopamine might function to encode information related to interval timing, defined as the duration of time required to organize a behavioral response, under conditions in which reward availability is temporally predictable (Buhusi and Meck, 2005; Matell et al., 2003; Meck, 1996). To directly test this theory, we compared changes in dopamine concentration during ICSS conditions in which cue-presentation was predictable (fixed time out = 10 s; FTO) versus conditions in which cue-presentation occurred variably (variable time out; = 30 s; VTO). As occurred in the ICSS-FTO task, reward availability was signaled to the animal by the presentation of a compound cue. This signaled reward availability across multiple sensory modalities; specifically, a house light turned off, an ongoing tone ceased and a white stimulus light mounted above the lever was presented. All stimuli were presented simultaneously with lever





**Figure 3. Disrupting Endocannabinoid Signaling Decreases Cue-Evoked Dopamine Neurotransmission during Appetitive Food Seeking**

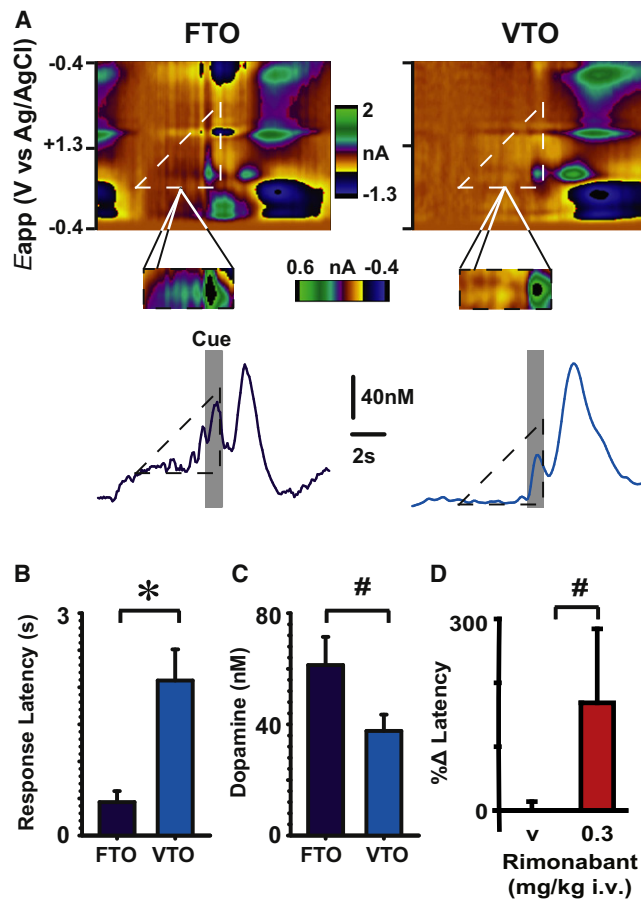
(A) Disrupting endocannabinoid signaling reduced reward seeking in a cued food self-administration task. Rimonabant dose-dependently (0.125 mg/kg i.v., orange bar; 0.3 mg/kg i.v., red bar) increased the latency to respond for food reinforcement in comparison to vehicle (v, blue bar). (B) Disrupting endocannabinoid transmission in the VTA specifically decreased appetitive rather than consummatory feeding behavior. Systemically (left, red bar), but not intrategmentally (right, red bar) administered CB1

receptor antagonists decreased intraoral intake of a chocolate Ensure solution in comparison to vehicle (v, blue bar), demonstrating that the VTA endocannabinoid system does not affect an animal's preferred meal size. (C) Diminished appetitive food seeking is accompanied by a decrease in mean dopamine concentration observed during the first second of cue-presentation following rimonabant (0.3 mg/kg i.v., red bar) treatment. (D) Representative color plots (top) and dopamine concentration traces (bottom) show the effects of rimonabant (right, red trace) in comparison to vehicle (left, blue trace) during individual trials. (E) A representative surface plot illustrates changes in dopamine concentration across trials (y axis) under baseline (black line), vehicle (blue line) and rimonabant (red line) conditions. (F) Disrupting endocannabinoid neurotransmission in the VTA is sufficient to decrease appetitive food seeking. Intrategmental rimonabant (200 ng i.c.; red bar) significantly increased response latency in comparison to vehicle (v, blue bar). (G) Rimonabant (200 ng i.c.; red bar) simultaneously decreased the mean cue-evoked dopamine concentration in comparison to vehicle treatment. (H) Representative traces illustrate that intrategmental rimonabant (200 ng i.c., right, red trace) decreased the concentration of cue-evoked dopamine in an individual trial in comparison to vehicle treatment (left, blue trace). Traces represent individual data, bars represent mean values, and error bars represent  $\pm$  SEM. A significant difference versus vehicle is indicated by either \* ( $p < 0.01$ ) or # ( $p < 0.05$ ).

extension. As predicted, anticipatory dopamine (Figure 4A) was only observed under FTO conditions. Importantly also, the concentration of cue-evoked dopamine was significantly lower under VTO conditions (Figure 4C; MWU test,  $U = 27.5$ ,  $p = 0.032$ ;  $n = 11$ ), which likely reflects a decrease in value imposed by the longer, unpredictable delays in reward availability occurring in the ICSS-VTO task (Bromberg-Martin and Hikosaka, 2011; Day et al., 2010; Kobayashi and Schultz, 2008), while response latencies were significantly increased (Figure 4B; MWU test,  $U = 24$ ,  $p < 0.01$ ;  $n = 14$ ) due to greater operandum disengagement. The data presented in Figure 2 demonstrate that rimonabant decreased cue-evoked dopamine signaling and reward seeking in the ICSS-FTO task. Under these conditions however, rather than decreasing reward-directed behavior by interfering with the neural representation of an environmental cue, disrupting endocannabinoid neurotransmission might decrease reward-directed behavior by interfering with an interoceptive timing signal because pharmacological manipulation of either the endocannabinoid or mesolimbic dopamine system can modulate neural representations of time during behavioral tasks (Crystal et al., 2003; Meck, 1983, 1996; Taylor et al., 2007). To address this, we tested the effects of rimonabant using the ICSS-VTO procedure. Rimonabant significantly increased the latency to respond in the ICSS-VTO task (Figure 4D; MWU test,  $U = 0$ ,  $p < 0.05$ ;  $n = 4$ ) as occurred in the ICSS-FTO task, thereby supporting our hypothesis that endocannabinoids regulate reward directed behavior by modulating the encoding of environmental cues predicting reward availability rather than interfering with interval timing.

### VDM11 Decreases Cue-Evoked Dopamine Concentrations and Reward Seeking

We next sought to assess the effects of augmenting endocannabinoid levels on the neural mechanisms of reward seeking. The



**Figure 4. Disrupting Endocannabinoid Signaling Decreases Reward Seeking under Experimental Conditions that Do Not Recruit Neural Mechanisms of Interval Timing**

(A) Transient dopamine concentrations increase during the anticipation of reward under conditions in which cue presentation is predictable (e.g., fixed timeout [FTO] = 10 s) but not under conditions in which cue presentations occur variably (variable timeouts [VTO] = 30 s). Representative mean (average of 30 trials/condition) color plots (top) and corresponding dopamine concentration traces (bottom) illustrate changes in dopamine concentration occurring prior to cue presentation from the same animal responding for brain stimulation reward under FTO (left) then VTO (right) conditions. Middle inset shows a magnification of the color plots showing dopamine concentration increases occurring prior to cue presentation.

(B) The mean latency to respond for brain stimulation reward was significantly greater under VTO (light blue bar) in comparison to FTO (dark blue bar) conditions.

(C) The mean dopamine concentration occurring during the first second of cue-presentation was significantly decreased under VTO in comparison the FTO conditions.

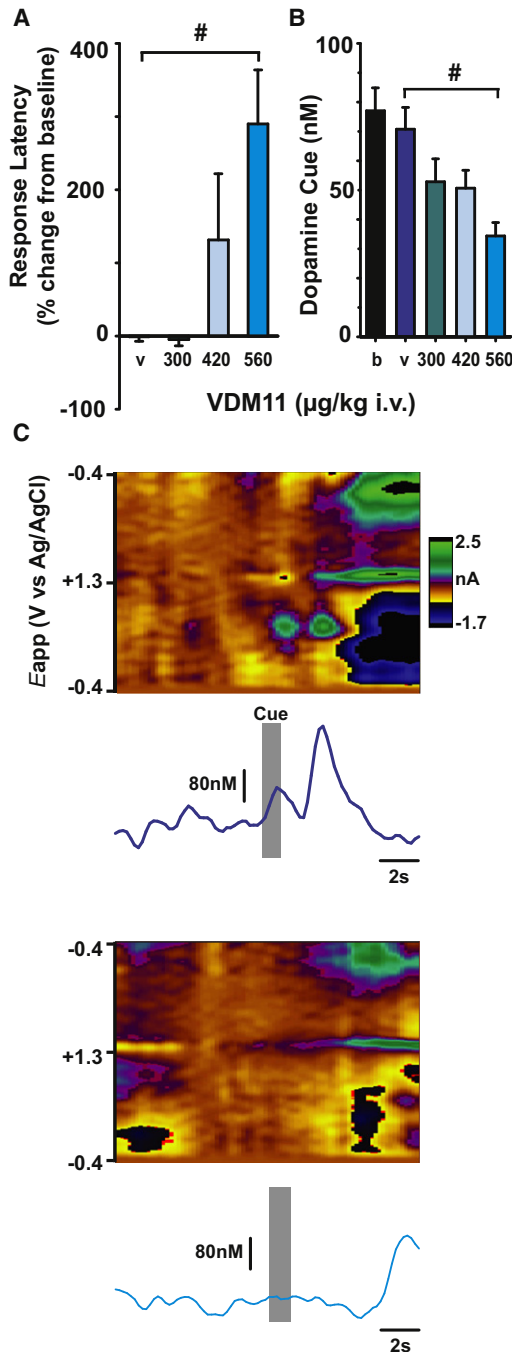
(D) Under VTO conditions, rimonabant (0.3 mg/kg i.v., red bar) significantly increased the latency to respond for brain stimulation reward in comparison to vehicle (v, blue bar). Traces and bars represent mean values while error bars represent  $\pm$  SEM. A significant difference versus vehicle is indicated by either \* ( $p < 0.01$ ) or # ( $p < 0.05$ ).

ICSS-VTO task was selected to eliminate potential floor effects involving response latency (as latencies to respond in the ICSS-FTO task can be in the subsecond range for well-trained animals). To increase endocannabinoid concentrations, animals

were treated with the putative endocannabinoid uptake inhibitor VDM11 using a cumulative dosing approach. Contrary to our hypotheses, VDM11 dose-dependently (300–560  $\mu$ g/kg i.v.) increased response latency (Figure 5A;  $F_{(2,23)} = 5.69$ ,  $p < 0.01$ ; 560  $\mu$ g/kg versus vehicle,  $p = 0.013$ ; mean values:  $b = 1.25$ ,  $v = 1.26$ ,  $0.1 = 1.28$ ,  $0.3 = 4.47$ ,  $0.56 = 5.16$  s) while decreasing the concentration of cue-evoked dopamine release in a manner similar to rimonabant (Figure 5B;  $F_{(4,29)} = 3.66$ ,  $p = 0.018$ ; 560  $\mu$ g/kg versus vehicle,  $p = 0.047$ ; also see Figure S3A for mean dopamine concentration traces). Figure 5C shows representative color plots and dopamine concentration traces illustrating the effects of vehicle (top) and VDM11 (bottom) in individual trials. These findings suggest that, under these conditions, VDM11 impairs the neural mechanisms of reward seeking by functioning as an indirect CB1 receptor antagonist.

### Repeated Vehicle Treatments Fail to Affect Cue-Evoked Dopamine Concentrations and Reward Seeking

In addition to observing drug-induced decreases in cue-evoked dopamine concentration however, we noted that the concentration of electrically-evoked dopamine also decreased across trials (Figure S1A for Rimonabant; Figure S3A for VDM11). This observation led us to test whether the decreases in cue- and electrically evoked dopamine concentration were drug-induced, or rather, the result of repeated vehicle injections occurring in prolonged ICSS sessions. To address this, we measured changes in NAc dopamine concentration and response latency for brain stimulation reward in the ICSS-VTO task while administering vehicle every 30 responses. Prior to ICSS-VTO session onset, animals were first trained to criterion in the ICSS-FTO task to mimic experimental conditions. Thus, rather than assessing dopamine-release events during acquisition (Figure 1), this experiment assessed dopamine concentrations over time as would occur during pharmacological experiments. Best-fit functions revealed that across trials cue-evoked dopamine concentrations quickly increased to an unvarying maximal level (Figure 6A; Exponential Rise to Maximum, Single, 2-Parameter;  $R^2 = 0.35$ ;  $F_{(1,19)} = 9.85$ ,  $p < 0.01$ ), while response latencies quickly decreased to an unvarying minimal level (Figure 6B; Polynomial, Inverse Second Order;  $R^2 = 0.25$ ;  $F_{(2,39)} = 6.08$ ,  $p < 0.01$ ). After the first 30 responses, both the concentration of cue-evoked dopamine and response latency remained statistically indistinguishable across binned responses. By contrast, electrically evoked dopamine concentrations showed greater variability and decreased linearly across trials (Figure 6A; Polynomial, Linear;  $R^2 = 0.31$ ;  $F_{(1,19)} = 7.90$ ,  $p < 0.01$ ). Representative mean color plots and accompanying dopamine concentration traces (Figure 6C) show dopamine concentrations changing across binned-responses. Identical trends were observed in untreated animals (data not shown). These observations are in agreement with previous reports (Garris et al., 1999; Nicolaysen et al., 1988; Owesson-White et al., 2008) that electrically evoked dopamine concentrations, but not cue-evoked dopamine concentrations or response strength, decrease during ICSS sessions—an effect that has been attributed to the depletion of a readily releasable pool of dopamine by electrical stimulation (Nicolaysen et al., 1988; Owesson-White et al., 2008; Yavich and Tiihonen, 2000). Moreover, these data demonstrate that



**Figure 5. The Putative Endocannabinoid Uptake Inhibitor VDM11 Decreases Dopamine Mechanisms of Reward Seeking in a Manner that Is Consistent with Indirect CB1 Receptor Antagonism**

(A) VDM11 dose-dependently (300–560 µg/kg i.v., cumulative) increased the latency to respond for brain stimulation reward in the ICSS-VTO task in comparison to vehicle (v).

(B) Dopamine concentration observed during the first second of cue presentation in the ICSS-VTO task under baseline (b), vehicle (v), or drug (VDM11 300–560 µg/kg i.v., cumulative) conditions. VDM11 dose-dependently decreased the mean concentration of cue-evoked dopamine.

(C) Representative color plots (top) and dopamine concentration traces (bottom) show the effects of VDM11 (560 µg/kg i.v., right, light blue line) and

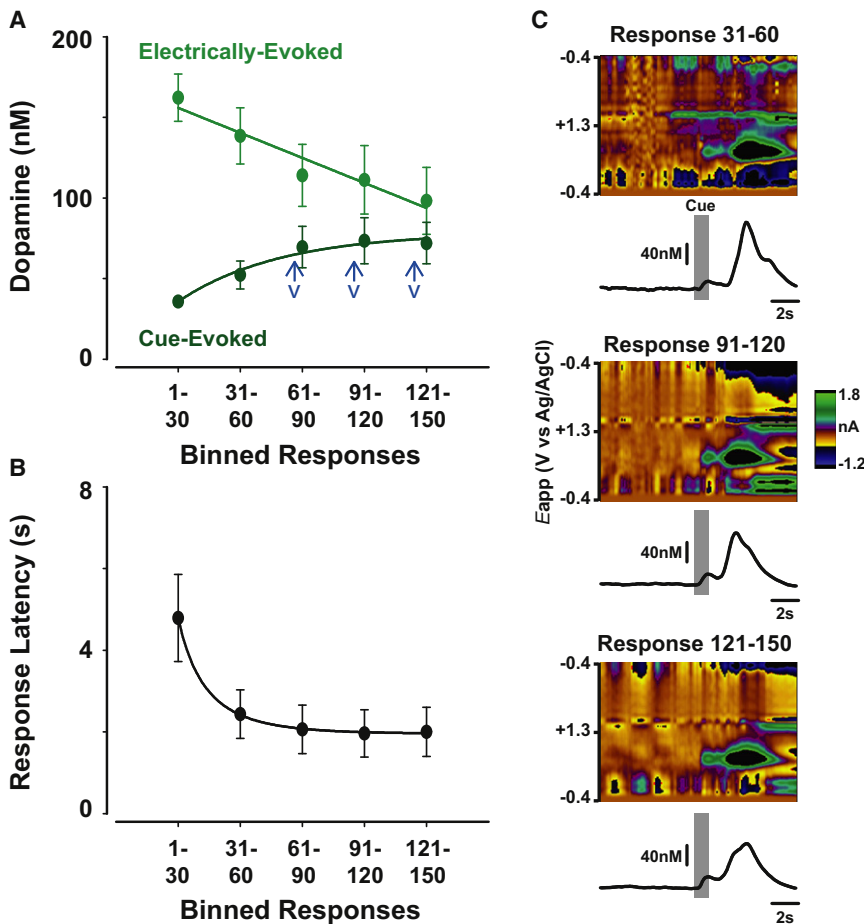
repeated vehicle injections fail to affect either cue-evoked dopamine concentrations or response latency. These findings, however, do not completely disprove that the endocannabinoid system might modulate electrically evoked dopamine release. The variables (e.g., route of administration, pharmacological target) that might influence the actions of endocannabinoids on electrically-evoked dopamine release should be further addressed.

**2AG but Not Anandamide Facilitates Reward Seeking**

The VDM11 findings prompted us to investigate the specific effects of the endocannabinoids 2AG and anandamide on reward seeking. 2AG and anandamide levels are tightly regulated through distinct enzymatic degradation systems. 2AG is hydrolyzed by the enzyme monoacylglycerol lipase (MAGL), whereas anandamide is hydrolyzed by the enzyme fatty acid amide hydrolase (FAAH) (Cravatt et al., 1996; Long et al., 2009). Recent advances in pharmacology have led to the development of drugs that selectively inhibit either MAGL (JZL184; Long et al., 2009) or FAAH (URB597; Cravatt et al., 1996; Fegley et al., 2005; thereby producing specific increases in 2AG or anandamide tissue levels, respectively. We began testing the effects of these drugs in mice because JZL184 is known to exhibit reduced potency against MAGL in rats (Long et al., 2009). In mice, JZL184 (Figure 7A;  $F_{(2,14)} = 6.61$   $p = 0.019$ ; 40 mg/kg versus vehicle,  $p = 0.029$ ), but not URB597 (data not shown), increased break points (a metric of motivation) for food reinforcement maintained under a progressive ratio schedule (Supplemental Experimental Procedures). Importantly, the JZL184-induced increase in break points was prevented by pretreating mice with a subthreshold dose of AM251 (0.75 mg/kg i.p.), which demonstrates that the JZL184-induced increase in motivation occurred in a CB1 receptor dependent manner. In rats, we observed increased break points (Figure 7A MWU test,  $U = 50.5$ ,  $p = 0.026$ ;  $n = 14$ ) for food reinforcement only after altering the route of administration and unit-injection dose (10 mg/kg JZL184 i.v.). Using a cumulative dosing approach, JZL184 (3–10 mg/kg i.v.) also facilitated reward seeking as assessed by decreased response latency in the ICSS-VTO task (Figure 7B;  $F_{(3,15)} = 4.86$   $p < 0.01$ ; 10 mg/kg versus vehicle,  $p = 0.027$ ; mean values: b = 4.02, v = 3.93, 3 = 3.83, 5.6 = 3.62, 10 = 4.32 s). By contrast, URB597 treatment (10–56 µg/kg i.v.) was ineffective at altering response latency (Figure 7C; mean values: b = 4.25, v = 4.19, 10 = 4.19, 31 = 6.15, 56 = 5.63 s) in the ICSS-VTO procedure, or break points for food reinforcement maintained under a progressive ratio schedule (Figure 7A). VDM11 (5.6 mg/kg i.v.) also failed to affect break points for food reinforcement (data not shown). To verify that JZL184 was indeed increasing activation of CB1 receptors, we treated rats with cumulative doses of JZL184 (5.6–10 mg/kg i.v.) while monitoring core body temperature as cannabinoids produce hypothermia in rats (Garattini, 1965;

vehicle (left, dark blue line) during individual trials of the ICSS-VTO task. Dopamine traces represent individual data, bars represent mean values and error bars represent ± SEM. A significant difference versus vehicle is indicated by # ( $p < 0.05$ ).





**Figure 6. Electrically Evoked, but Not Cue-Evoked, Dopamine Concentrations Are Partially Depleted during ICSS Sessions**

(A) Electrically (light green) and cue (dark green)-evoked dopamine concentrations across binned responses. Animals are responding for brain stimulation reward in the ICSS-VTO task during repeated vehicle (1:1:18, v, blue arrow) treatments. Under these conditions, cue-evoked dopamine concentrations initially increased to maximum and then remained stable throughout the duration of the session. Conversely, electrically evoked dopamine concentrations decreased linearly across the duration of the session. (B) Corresponding response latency values. The latency to respond for brain stimulation reward initially decreased to minimum and then remained stable throughout the duration of the session. (C) Representative mean color plots (top) and corresponding dopamine concentration traces (bottom) illustrate changes in dopamine concentration occurring across binned responses. Nonlinear regression functions show best-fit data trends. Data points and dopamine traces represent mean data, and error bars represent  $\pm$  SEM.

signaling during individual trials is illustrated by the representative color plots and accompanying dopamine concentration traces (Figure 8C), while the effect of JZL184 on dopamine signaling across trials is shown by the representative surface plot (Figure 8D). To confirm that 2AG levels within the VTA are alone sufficient to facilitate the neural mechanisms

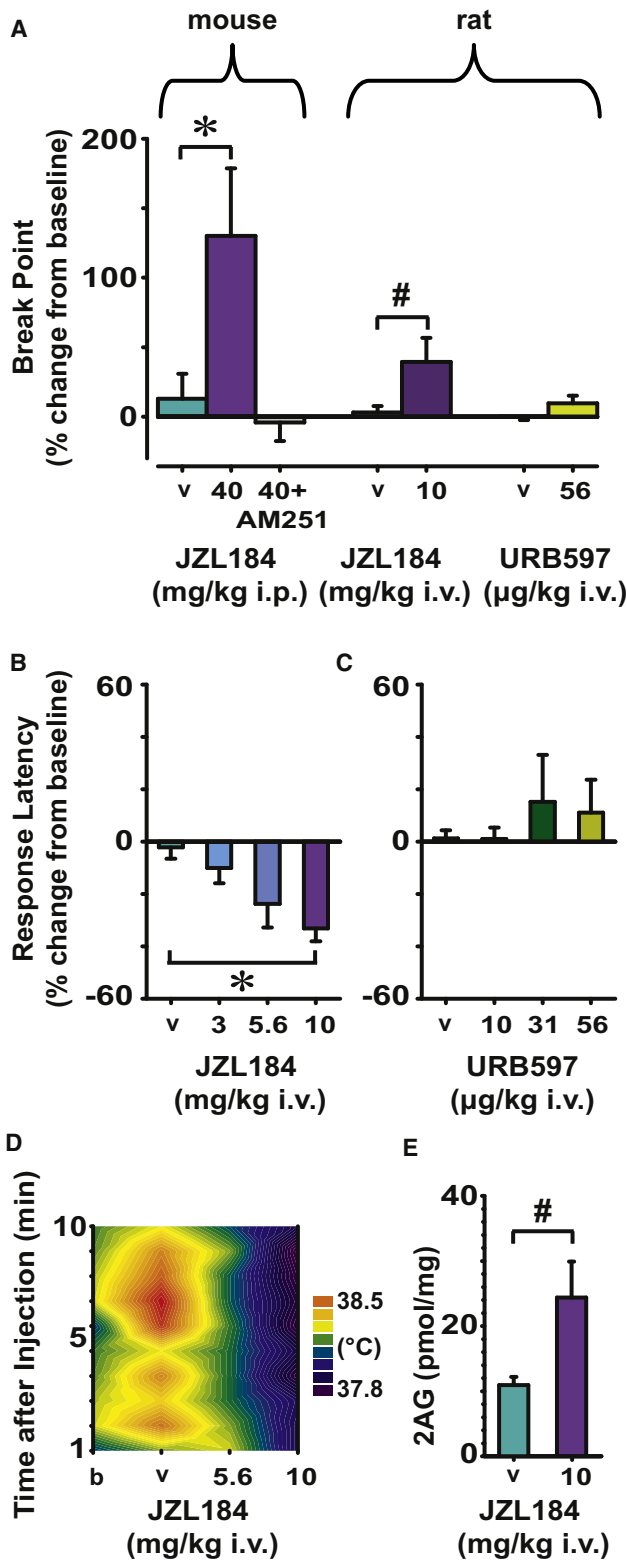
Miras, 1965). As predicted (Long et al., 2009), JZL184 decreased core temperature across time (Figure 7D;  $F_{(3,34)} = 2.63$ ,  $p < 0.01$ ). To definitively test whether JZL184 increases 2AG levels during reward seeking, we assessed lipid content in VTA tissue from JZL184 and vehicle-treated rats upon completion of the ICSS-VTO task and found that JZL184 significantly increased 2AG VTA tissue content in comparison to vehicle (Figure 7E;  $t_{(27)} = 2.07$ ,  $p = 0.048$ ), thereby confirming that JZL184 augments 2AG levels in the VTA during reward directed behavior in the rat.

#### Increasing 2AG Levels Facilitates Cue-Evoked Dopamine Release and Reward Seeking

To assess the effects of increasing 2AG levels on the neural mechanisms of reward seeking we treated rats with JZL184 (10 mg/kg i.v.) while responding was maintained by brain stimulation reward in the ICSS-VTO task. As observed using a cumulative dosing approach, JZL184 (10mg/kg i.v.) decreased response latency (Figure 8A;  $t_{(14)} = 2.36$ ,  $p = 0.033$ ; mean values:  $b = 3.55$ ,  $v = 3.48$ , JZL = 2.89 s). Enhanced reward seeking occurred in parallel with an increase in cue-evoked dopamine concentration (Figure 8B;  $F_{(2,14)} = 10.86$   $p < 0.01$ ; 10 mg/kg versus vehicle,  $p < 0.01$ ; also see Figure S3B for mean dopamine concentration traces). The effect of JZL184 on dopamine

of reward seeking, we infused JZL184 into the VTA while measuring dopamine concentrations and behavior maintained in the ICSS-VTO task. Although the required vehicle to achieve solubility (a 6  $\mu$ g/0.5  $\mu$ l solution required 100% dimethyl sulfoxide [DMSO]) increased response latency; remarkably, intrategmental JZL184 (6  $\mu$ g, ipsilateral) reversed the DMSO-induced deficits in reward seeking (Figure 8E;  $t_{(6)} = -2.51$ ,  $p = 0.046$ ; mean values:  $b = 3.75$ , DMSO = 4.61, JZL = 3.47 s) while increasing cue-evoked dopamine concentrations (Figure 8F;  $F_{(2,18)} = 10.84$   $p < 0.01$ ; 6  $\mu$ g versus vehicle,  $p = 0.023$ ). To verify that the effects of intrategmental JZL184 on reward seeking were CB1 receptor dependent, we then treated rats with a subthreshold dose of rimonabant (1.25 mg/kg i.v.), which reverted response latencies to DMSO conditions. The effects of intrategmental DMSO and JZL184 on cue evoked dopamine events occurring in individual trials are illustrated by the representative traces in Figure 8G, whereas the effects across trials are depicted in a representative surface plot (Figure S4C). JZL184-induced increases in cue-evoked dopamine concentration and reward seeking can also be observed by viewing audio-visual material (Movie S3). Taken together, these data suggest that augmenting 2AG within the VTA is sufficient to facilitate mesolimbic dopaminergic mechanisms of reward seeking.





**Figure 7. The Endocannabinoid 2AG, but Not Anandamide, Facilitates Reward Seeking**

(A) The effects of JZL184 (decreases degradation of 2AG) or URB597 (decreases degradation of anandamide) on break points for food reinforcement.

**DISCUSSION**

It is well documented that transient dopamine concentrations in the NAc encode information regarding motivationally salient stimuli that predict reward availability (Day et al., 2007; Flagel et al., 2011; Phillips et al., 2003). Little is known however, regarding how these transient increases are modulated at dopamine cell bodies within the VTA. In the present study, we used a cutting-edge electrochemical monitoring technique to investigate how endocannabinoids in the VTA modulate transient dopamine release into the NAc shell during reward seeking. We found that disrupting endocannabinoid modulation of dopamine neurons reduced cue-evoked dopamine concentrations and reward seeking. Moreover, we identified that 2AG, rather than anandamide, is the primary endocannabinoid responsible for facilitating the neural mechanisms of reward seeking. Thus, our findings reveal that the VTA endocannabinoid system is critical for the fine-tuned regulation of dopamine signaling that mediates reward-directed behavior.

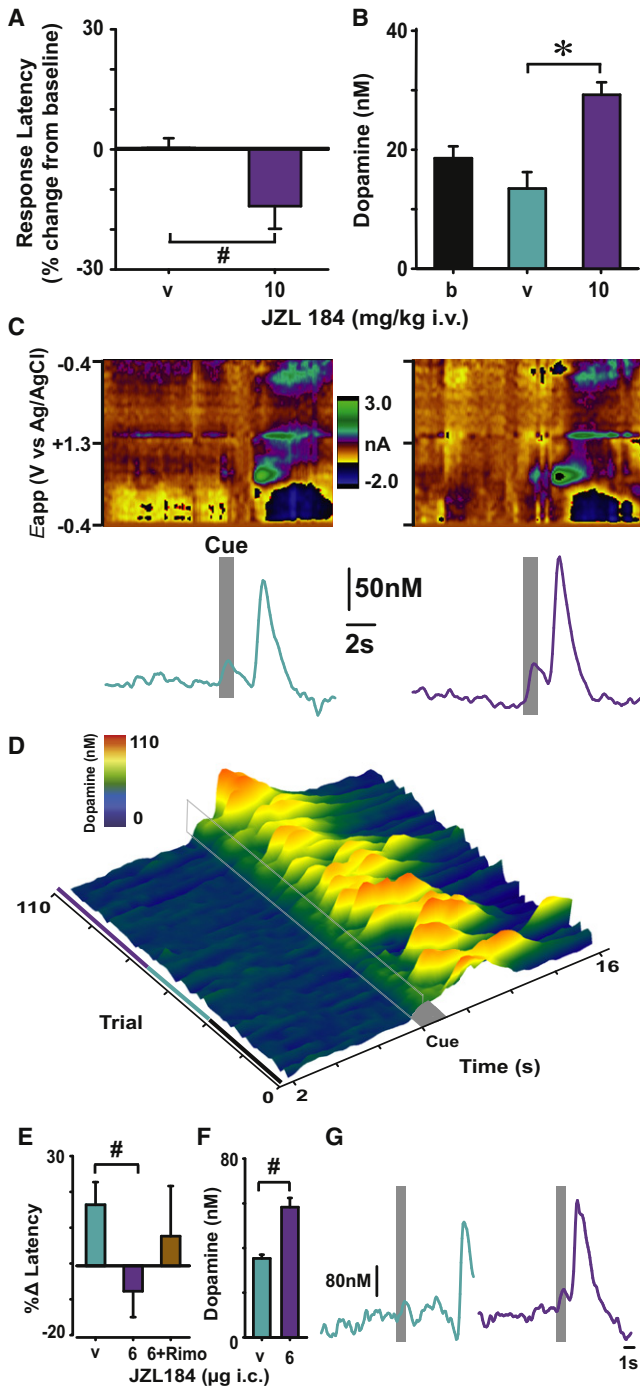
Our data demonstrate the existence of a single neural signaling mechanism through which CB1 antagonists can effectively diminish the influence that environmental cues exert over motivated behavior. A number of studies have shown that the endocannabinoid system is involved in the appetitive-motivational aspects of reward-directed behavior. For example, motivation for both palatable foods (Ward and Dykstra, 2005) and drugs of abuse (Solinas et al., 2003; Xi et al., 2008) is decreased by pharmacological disruption of endocannabinoid signaling as assessed by break points under a progressive ratio schedule. A current theory holds that endocannabinoids are specifically involved in modulating the secondary/environmental influences on motivated behavior (Le Foll and Goldberg, 2004; De Vries and Schoffelmeer, 2005). In support of this view, when operant behavior is maintained by conditioned cues (i.e., under a second order schedule), pharmacological disruption of endocannabinoid signaling decreases responding (Justinova et al., 2008). Moreover, endocannabinoid disruption is particularly effective at reducing cue-induced reinstatement, a model of relapse in humans that incorporates the influence of conditioned environmental stimuli on reward seeking (Epstein et al., 2006). In this

ment maintained under a progressive ratio schedule. Left: In mice, JZL184 (40 mg/kg i.p., purple bar) increased break points for food reinforcement in comparison to vehicle (v, green bar). Pretreatment with a subthreshold dose of AM251 (0.75 mg/kg i.p.) prevented the JZL184-induced increase in break point. Middle: In rats, JZL184 (10 mg/kg i.v.) produced a significant increase in mean final ratio when compared to vehicle. Right: In rats, URB597 (56 µg/kg i.v., yellow bar) failed to increase the break points for food.

(B and C) JZL184 (3–10 mg/kg i.v. cumulative) but not URB597 (10–56 µg/kg i.v., cumulative) decreased response latency for brain stimulation reward in the ICSS-VTO task in rats.

(D) Topographic plot showing core temperature of rats (z axis) over time (y axis) under baseline (b), vehicle (v), and JZL184 (5.6 then 10 mg/kg i.v. cumulative) conditions.

(E) JZL184 (10 mg/kg i.v., purple bar) treated rats showed a significant increase in 2AG VTA tissue content in comparison to vehicle treated rats. Tissue samples were collected immediately after ICSS-VTO sessions. Bars represent mean ± SEM values. A significant difference versus vehicle is indicated by either \* (p < 0.01) or # (p < 0.05).



**Figure 8. The Endocannabinoid 2AG Facilitates Dopaminergic Mechanisms of Reward Seeking**

(A) Augmenting 2AG levels facilitated reward seeking in the ICSS-VTO task. JZL184 (10 mg/kg i.v., purple bar) decreased response latency in comparison to vehicle (v, blue bar).  
 (B) Facilitated reward seeking was accompanied by an increase in cue-evoked dopamine concentration.  
 (C) Representative color plots (top) and dopamine concentration traces (bottom) show the effects of JZL184 (right, purple trace) in comparison to vehicle (left, green trace) during individual trials.

model, CB1 receptor antagonists decrease the propensity for conditioned cues to reinstate responding for appetitive food (Ward et al., 2007) and various drugs of abuse (Justinova et al., 2008; De Vries and Schoffelmeer, 2005). Importantly, the finding that disrupting endocannabinoid signaling decreases reward seeking regardless of the reinforcer paired with the cue (De Vries and Schoffelmeer, 2005) implies that a common neural mechanism is involved through which endocannabinoids regulate cue-motivated behavior. Our data suggest that this common neural mechanism involves endocannabinoid disinhibition of cue-evoked dopamine cell firing in the VTA, as pharmacological disruption of endocannabinoid signaling within this brain region was sufficient to decrease cue-evoked dopamine concentrations and reward seeking behavior in unison. It is likely that following systemic administration of CB1 receptor antagonists; however, diminished surges in dopamine concentration interact with altered accumbal glutamate concentrations (Xi et al., 2008), possibly arising from the prefrontal cortex (Alvarez-Jaimes et al., 2008), to decrease reward seeking. Such an interaction would be consistent with the theory that accumbal dopamine affects reward seeking by modulating convergent cortical, hippocampal, and amygdalar input (Brady and O'Donnell, 2004; Floresco et al., 2001). Furthermore, CB1 receptors within the NAc likely contribute to decreased reward seeking following systemic administration of CB1 receptor antagonists (Alvarez-Jaimes et al., 2008). Nevertheless, our findings that intrategmental disruption of endocannabinoid signaling alone simultaneously decreased cue-evoked dopamine concentrations and reward seeking suggests that the VTA endocannabinoid system is critically involved in mediating cue-motivated reward-directed behavior.

We therefore predicted that increasing endocannabinoid levels would facilitate the neural mechanisms of reward seeking. VDM11 however, dose-dependently decreased cue-evoked dopamine signaling and reward seeking in a manner that is more consistent with VDM11 reducing presynaptic CB1 receptor activation. These findings are in agreement with recent reports demonstrating that endocannabinoid uptake inhibitors can decrease cue-induced reinstatement of drug-seeking behavior in a manner similar to rimonabant when assessed using self-administration (Gamaledin et al., 2011) or conditioned place preference paradigms (Scherma et al., 2012). One possible mechanism explaining these findings is that VDM11 decreases

(D) A representative surface plot illustrates changes in dopamine concentration across trials (y axis) under baseline (black line), vehicle (green line), and rimonabant (purple line) conditions.

(E) Augmenting 2AG in the VTA is sufficient to facilitate reward seeking. JZL184 (6 μg, ipsilateral, purple bar) decreased response latency in comparison to DMSO (green bar). Posttreatment with a subthreshold dose of rimonabant (1.25 mg/kg i.v.) reversed the JZL184-induced decrease in reward latency.

(F) Facilitated reward seeking occurred simultaneously with an increase in cue-evoked dopamine concentration in comparison to vehicle.

(G) Representative traces show the effects of intrategmental vehicle (left, green trace) and JZL184 (right, purple trace) on cue-evoked dopamine concentration in individual trials. Traces represent individual data, bars represent mean values, and error bars represent  $\pm$  SEM. A significant difference versus vehicle is indicated by either \* ( $p < 0.01$ ) or # ( $p < 0.05$ ).

CB1 receptor activation by interfering with the bidirectional release of endocannabinoids through a putative transport mechanism (Hillard et al., 1997; Melis et al., 2004; Ronesi et al., 2004). Another mechanistic explanation is that VDM11 might selectively increase anandamide (van der Stelt et al., 2006), which could function as a competitive antagonist at CB1 receptors in the presence of 2AG because, in contrast to 2AG, anandamide is a partial agonist at CB1 receptors (Howlett and Mukhopadhyay, 2000). These findings led us to investigate the respective contributions of 2AG and anandamide. 2AG, but not anandamide, increased motivation, reward seeking, and cue-evoked dopamine concentrations. These data demonstrate that 2AG is the primary endocannabinoid that enhances the neural mechanisms of cue-motivated reward seeking and agree with reports demonstrating that 2AG is the principal endocannabinoid for multiple forms of synaptic plasticity across several brain regions (Melis et al., 2004; Tanimura et al., 2010).

Based on our data, we speculate that 2AG might modulate cue-evoked dopamine release through disinhibition of dopamine neurons in the VTA. When dopamine neurons fire at high frequencies they release 2AG (Melis et al., 2004), which then retrogradely binds to CB1 receptors on presynaptic terminals within the VTA (Lupica and Riegel, 2005). Although 2AG would affect both GABAergic and glutamatergic synaptic input through CB1 receptor activation (Mátyás et al., 2008)—cue-encoding VTA dopamine neurons are theorized to form discrete neural assemblies with GABAergic synapses, thereby allowing for the fine-tuned regulation of dopamine neural activity during reward seeking (Lupica and Riegel, 2005; Mátyás et al., 2008). According to this conceptualization, 2AG activation of CB1 receptors located on GABAergic terminals might decrease GABA release onto VTA dopamine neurons. The reduced GABA tone theoretically would decrease activation of GABA receptors on VTA dopamine neurons, thus resulting in a disinhibition of dopamine neural activity (Lupica and Riegel, 2005). The resulting disinhibition of dopamine neural activity is theorized to facilitate the neural mechanisms of reward seeking. It is important to clarify that using this freely moving recording approach, other mechanisms within the VTA may account for the observed findings.

We further speculate that endocannabinoid modulation of dopamine release from the VTA might affect NAc neural activity through a D1 receptor dependent mechanism. While recent evidence indicates that dopamine does not directly change postsynaptic excitability in the NAc (Stuber et al., 2010; Tecuapetla et al., 2010), it remains well accepted that dopamine can modulate input into the striatum, as occurs during reward seeking, to affect neural responses in a D1 receptor dependent manner (Cheer et al., 2007a; Goto and Grace, 2005; Reynolds et al., 2001). It is possible therefore, that the VTA endocannabinoid system might affect NAc neural activity by increasing D1 receptor occupancy. Recently developed computational models of dopamine signaling offer insight into how dopamine transients might influence NAc neural activity specifically through a D1 receptor-mediated mechanism (Dreyer et al., 2010). When dopamine neurons exhibit regular pacemaker firing, low concentrations (i.e., tonic) of dopamine are released throughout the NAc (Floresco et al., 2003). The computational model predicts

that during tonic dopamine signaling, D2 receptors approach maximal occupancy whereas D1 receptors remain relatively unaffected (Dreyer et al., 2010). By contrast, when dopamine neurons fire at high frequency, transient bursts of dopamine are heterogeneously released into discrete microcircuits of the NAc (Dreyer et al., 2010; Wightman et al., 2007). When these higher concentration transients occur—D1 receptor occupancy theoretically increases precipitously whereas D2 receptors, which are already approaching maximal occupancy, remain relatively unaffected (Dreyer et al., 2010). Thus, we hypothesize that endocannabinoid disruption in the VTA might decrease NAc neural activity by preventing sufficient D1 receptor occupancy.

The present study offers previously unseen insights regarding the neural mechanisms underlying reward seeking motivated by conditioned cues. Our data demonstrate for the first time that 2AG within the VTA can modulate cue-evoked dopamine transients, which are theorized to promote reward seeking (Nicola, 2010; Phillips et al., 2003). While we (Cheer et al., 2007b) and others (Perra et al., 2005) have demonstrated that disrupting the VTA endocannabinoid system decreases drug-induced dopamine release, this is the first demonstration that the endocannabinoid system modulates cue-evoked dopamine transients during the pursuit of reward. Furthermore, our data suggest that drugs designed to specifically manipulate 2AG levels may prove to be effective pharmacotherapies for the treatment of neuropsychiatric disorders involving a maladaptive motivational state.

## EXPERIMENTAL PROCEDURES

### Subjects

Male Sprague-Dawley rats, ~90–120 days old (300–350 g), fitted with back mounted jugular vein catheters at vendor (Charles River) were used as subjects. Subjects were anesthetized with isoflurane (5% isoflurane induction, 2% maintenance) in a Kopf stereotaxic apparatus and implanted with a microdialysis guide cannula (BAS) aimed at the NAc shell (+1.7 AP, +0.8 ML), an ipsilateral bipolar stimulating electrode (Plastics One) in the VTA (−5.4 AP, +0.5 ML, −8.7 DV), and a contralateral Ag/AgCl reference electrode. All procedures were performed in accordance to the University of Maryland, Baltimore's Institutional Animal Care and Use Committee protocols.

### Fast-Scan Cyclic Voltammetry

Dopamine was detected from fast-scan cyclic voltammograms collected at the carbon fiber electrode every 100 ms (initial waveform: −0.4V to 1.3V, 400V/s [Heien et al., 2003]). Principal component regression (PCR) was used as previously described to extract the dopamine component from the raw voltammetric data (Heien et al., 2005).

### Dopamine Signal Calibration

Principal component regression (PCR) was used as previously described to extract the dopamine component from the raw voltammetric data (Heien et al., 2005). A calibration set of stimulations was obtained for each experiment varying number of stimulation pulses (6, 12, or 24) and frequency (30 or 60 Hz). Scaling factors for both DA and pH were obtained post experiment by placing the electrode into a flow injection system and injecting known concentrations of DA and pH into artificial cerebrospinal fluid. These scaling factors related current values to concentration values.

### Microinfusions

For experiments involving intrategmental infusions, rats were unilaterally treated with vehicle (DMSO; 0.5  $\mu$ l), rimonabant 200 ng/0.5  $\mu$ l or JZL184

6  $\mu\text{g}/0.5 \mu\text{l}$ . Infusions occurred in the experimental chamber using a microprocessor-controlled infusion pump (Harvard Apparatus). An infusion needle was inserted through a guide cannula ending 1 mm above the tip of a bilateral stimulating electrode (Plastics One); the needle was cut to extend 1 mm beyond the cannula tip.

### Histology

Rats were placed under deep anesthesia (2 mg/kg urethane). A high amplitude current (500  $\mu\text{A}$ ) was applied through a stainless steel electrode to verify working electrode placement. Rats were then intracardially perfused with saline, potassium ferrocyanide stain, and 10% formalin. Brains were removed, cryoprotected, and coronally sectioned using a cryostat. See Figure S5 for representative illustrations confirming electrode placement.

### Statistics

Behavioral analyses were statistically evaluated using the Shapiro-Wilk test for normality. If not normally distributed, data were analyzed with either the Mann-Whitney U (MWU) test or Kruskal-Wallis ANOVA on ranks. If normally distributed, data were analyzed with either the Student's t test or ANOVA. Dopamine concentrations occurring during the first second of cue presentation were analyzed with ANOVA and Bonferroni post-hoc tests. All statistical analyses were performed with SigmaPlot (version 11).

### SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures, three movies, and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.neuron.2011.11.018.

### ACKNOWLEDGMENTS

Funding for this study was provided by NIH grants R01DA022340 (J.F.C.), R01DA025634 (M.F.R.), P01DA009789 (A.H.L.), F32DA032266 and T32NS007375 (E.B.O.), and a Rubicon Fellowship (C.S.L.). We also thank Geoff Schoenbaum, Peter Shizgal, Yolanda Mateo, and Joshua Jones for helpful comments in the preparation of this manuscript, John Peterson for instrumentation assistance and Merce Masana, Niels Vos, Ronny Gentry, and David Bernstein for technical assistance.

Accepted: November 15, 2011

Published: January 25, 2012

### REFERENCES

Alvarez-Jaimes, L., Polis, I., and Parsons, L.H. (2008). Attenuation of cue-induced heroin-seeking behavior by cannabinoid CB1 antagonist infusions into the nucleus accumbens core and prefrontal cortex, but not basolateral amygdala. *Neuropsychopharmacology* 33, 2483–2493.

Berridge, K.C., and Robinson, T.E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res. Brain Res. Rev.* 28, 309–369.

Berry, E.M., and Mechoulam, R. (2002). Tetrahydrocannabinol and endocannabinoids in feeding and appetite. *Pharmacol. Ther.* 95, 185–190.

Bindra, D. (1968). Neuropsychological interpretation of the effects of drive and incentive-motivation on general and instrumental behavior. *Psychol. Rev.* 75, 1–22.

Brady, A.M., and O'Donnell, P. (2004). Dopaminergic modulation of prefrontal cortical input to nucleus accumbens neurons in vivo. *J. Neurosci.* 24, 1040–1049.

Bromberg-Martin, E.S., and Hikosaka, O. (2011). Lateral habenula neurons signal errors in the prediction of reward information. *Nat. Neurosci.* 14, 1209–1216.

Buhusi, C.V., and Meck, W.H. (2005). What makes us tick? Functional and neural mechanisms of interval timing. *Nat. Rev. Neurosci.* 6, 755–765.

Cheer, J.F., Aragona, B.J., Heien, M.L., Seipel, A.T., Carelli, R.M., and Wightman, R.M. (2007a). Coordinated accumbal dopamine release and neural activity drive goal-directed behavior. *Neuron* 54, 237–244.

Cheer, J.F., Wassum, K.M., Sombers, L.A., Heien, M.L., Ariansen, J.L., Aragona, B.J., Phillips, P.E., and Wightman, R.M. (2007b). Phasic dopamine release evoked by abused substances requires cannabinoid receptor activation. *J. Neurosci.* 27, 791–795.

Cravatt, B.F., Giang, D.K., Mayfield, S.P., Boger, D.L., Lerner, R.A., and Gilula, N.B. (1996). Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* 384, 83–87.

Crystal, J.D., Maxwell, K.W., and Hohmann, A.G. (2003). Cannabinoid modulation of sensitivity to time. *Behav. Brain Res.* 144, 57–66.

Day, J.J., Roitman, M.F., Wightman, R.M., and Carelli, R.M. (2007). Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. *Nat. Neurosci.* 10, 1020–1028.

Day, J.J., Jones, J.L., Wightman, R.M., and Carelli, R.M. (2010). Phasic nucleus accumbens dopamine release encodes effort- and delay-related costs. *Biol. Psychiatry* 68, 306–309.

De Vries, T.J., and Schoffelmeier, A.N. (2005). Cannabinoid CB1 receptors control conditioned drug seeking. *Trends Pharmacol. Sci.* 26, 420–426.

Dreyer, J.K., Herrik, K.F., Berg, R.W., and Hounsgaard, J.D. (2010). Influence of phasic and tonic dopamine release on receptor activation. *J. Neurosci.* 30, 14273–14283.

Epstein, D.H., Preston, K.L., Stewart, J., and Shaham, Y. (2006). Toward a model of drug relapse: an assessment of the validity of the reinstatement procedure. *Psychopharmacology (Berl.)* 189, 1–16.

Fegley, D., Gaetani, S., Duranti, A., Tontini, A., Mor, M., Tarzia, G., and Piomelli, D. (2005). Characterization of the fatty acid amide hydrolase inhibitor cyclohexyl carbamic acid 3'-carbamoyl-biphenyl-3-yl ester (URB597): effects on anandamide and oleylethanolamide deactivation. *J. Pharmacol. Exp. Ther.* 313, 352–358.

Flagel, S.B., Clark, J.J., Robinson, T.E., Mayo, L., Czuj, A., Willuhn, I., Akers, C.A., Clinton, S.M., Phillips, P.E., and Akil, H. (2011). A selective role for dopamine in stimulus-reward learning. *Nature* 469, 53–57.

Floresco, S.B., Blaha, C.D., Yang, C.R., and Phillips, A.G. (2001). Modulation of hippocampal and amygdalar-evoked activity of nucleus accumbens neurons by dopamine: cellular mechanisms of input selection. *J. Neurosci.* 21, 2851–2860.

Floresco, S.B., West, A.R., Ash, B., Moore, H., and Grace, A.A. (2003). Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. *Nat. Neurosci.* 6, 968–973.

Foltin, R.W., and Haney, M. (2007). Effects of the cannabinoid antagonist SR141716 (rimonabant) and d-amphetamine on palatable food and food pellet intake in non-human primates. *Pharmacol. Biochem. Behav.* 86, 766–773.

Freund, T.F., Katona, I., and Piomelli, D. (2003). Role of endogenous cannabinoids in synaptic signaling. *Physiol. Rev.* 83, 1017–1066.

Gamaledin, I., Guranda, M., Goldberg, S.R., and Lefoll, B. (2011). The selective anandamide transport inhibitor VDM11 attenuates reinstatement of nicotine seeking induced by nicotine associated cues and nicotine priming, but does not affect nicotine-intake. *Br. J. Pharmacol.* 164, 1652–1660.

Garattini, S. (1965). Effects of a cannabis extract on gross behavior. In *Hashish: Its Chemistry and Pharmacology*, G. Ioakeimoglou, G.E.W. Wolstenholme, and J. Knight, eds. (Boston: Brown), pp. 70–82.

Garris, P.A., Kilpatrick, M., Bunin, M.A., Michael, D., Walker, Q.D., and Wightman, R.M. (1999). Dissociation of dopamine release in the nucleus accumbens from intracranial self-stimulation. *Nature* 398, 67–69.

Gomez, R., Navarro, M., Ferrer, B., Trigo, J.M., Bilbao, A., Del Arco, I., Cippitelli, A., Nava, F., Piomelli, D., and Rodriguez de Fonseca, F. (2002). A peripheral mechanism for CB1 cannabinoid receptor-dependent modulation of feeding. *J. Neurosci.* 22, 9612–9617.

Goto, Y., and Grace, A.A. (2005). Dopaminergic modulation of limbic and cortical drive of nucleus accumbens in goal-directed behavior. *Nat. Neurosci.* 8, 805–812.



- Heien, M.L., Phillips, P.E., Stuber, G.D., Seipel, A.T., and Wightman, R.M. (2003). Overoxidation of carbon-fiber microelectrodes enhances dopamine adsorption and increases sensitivity. *Analyst (Lond.)* 128, 1413–1419.
- Heien, M.L., Khan, A.S., Ariansen, J.L., Cheer, J.F., Phillips, P.E., Wassum, K.M., and Wightman, R.M. (2005). Real-time measurement of dopamine fluctuations after cocaine in the brain of behaving rats. *Proc. Natl. Acad. Sci. USA* 102, 10023–10028.
- Hillard, C.J., Edgmond, W.S., Jarrahan, A., and Campbell, W.B. (1997). Accumulation of N-arachidonylethanolamine (anandamide) into cerebellar granule cells occurs via facilitated diffusion. *J. Neurochem.* 69, 631–638.
- Howlett, A.C., and Mukhopadhyay, S. (2000). Cellular signal transduction by anandamide and 2-arachidonoylglycerol. *Chem. Phys. Lipids* 108, 53–70.
- Julian, M.D., Martin, A.B., Cuellar, B., Rodriguez De Fonseca, F., Navarro, M., Moratalla, R., and Garcia-Segura, L.M. (2003). Neuroanatomical relationship between type 1 cannabinoid receptors and dopaminergic systems in the rat basal ganglia. *Neuroscience* 119, 309–318.
- Justinova, Z., Munzar, P., Panlilio, L.V., Yasar, S., Redhi, G.H., Tanda, G., and Goldberg, S.R. (2008). Blockade of THC-seeking behavior and relapse in monkeys by the cannabinoid CB1-receptor antagonist rimonabant. *Neuropsychopharmacology* 33, 2870–2877.
- Kelley, A.E. (1999). Neural integrative activities of nucleus accumbens subregions in relation to learning and motivation. *Psychobiology* 27, 198–213.
- Kobayashi, S., and Schultz, W. (2008). Influence of reward delays on responses of dopamine neurons. *J. Neurosci.* 28, 7837–7846.
- Le Foll, B., and Goldberg, S.R. (2004). Rimonabant, a CB1 antagonist, blocks nicotine-conditioned place preferences. *Neuroreport* 15, 2139–2143.
- Long, J.Z., Li, W., Booker, L., Burston, J.J., Kinsey, S.G., Schlosburg, J.E., Pavón, F.J., Serrano, A.M., Selley, D.E., Parsons, L.H., et al. (2009). Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. *Nat. Chem. Biol.* 5, 37–44.
- Lupica, C.R., and Riegel, A.C. (2005). Endocannabinoid release from midbrain dopamine neurons: a potential substrate for cannabinoid receptor antagonist treatment of addiction. *Neuropharmacology* 48, 1105–1116.
- Matell, M.S., Meck, W.H., and Nicolelis, M.A. (2003). Interval timing and the encoding of signal duration by ensembles of cortical and striatal neurons. *Behav. Neurosci.* 117, 760–773.
- Mátyás, F., Urbán, G.M., Watanabe, M., Mackie, K., Zimmer, A., Freund, T.F., and Katona, I. (2008). Identification of the sites of 2-arachidonoylglycerol synthesis and action imply retrograde endocannabinoid signaling at both GABAergic and glutamatergic synapses in the ventral tegmental area. *Neuropharmacology* 54, 95–107.
- Meck, W.H. (1983). Selective adjustment of the speed of internal clock and memory processes. *J. Exp. Psychol. Anim. Behav. Process.* 9, 171–201.
- Meck, W.H. (1996). Neuropharmacology of timing and time perception. *Brain Res. Cogn. Brain Res.* 3, 227–242.
- Melis, M., Pistis, M., Perra, S., Muntoni, A.L., Pillolla, G., and Gessa, G.L. (2004). Endocannabinoids mediate presynaptic inhibition of glutamatergic transmission in rat ventral tegmental area dopamine neurons through activation of CB1 receptors. *J. Neurosci.* 24, 53–62.
- Miras, C. (1965). Some aspects of cannabis action. In *Hashish: Its Chemistry and Pharmacology*, G. Ioakeimoglou, G.E.W. Wolstenholme, and J. Knight, eds. (Boston: Brown), pp. 37–52.
- Mogenson, G.J., Jones, D.L., and Yim, C.Y. (1980). From motivation to action: functional interface between the limbic system and the motor system. *Prog. Neurobiol.* 14, 69–97.
- Nesse, R.M., and Berridge, K.C. (1997). Psychoactive drug use in evolutionary perspective. *Science* 278, 63–66.
- Nicola, S.M. (2010). The flexible approach hypothesis: unification of effort and cue-responding hypotheses for the role of nucleus accumbens dopamine in the activation of reward-seeking behavior. *J. Neurosci.* 30, 16585–16600.
- Nicolaysen, L.C., Ikeda, M., Justice, J.B., Jr., and Neill, D.B. (1988). Dopamine release at behaviorally relevant parameters of nigrostriatal stimulation: effects of current and frequency. *Brain Res.* 460, 50–59.
- Owesson-White, C.A., Cheer, J.F., Beyene, M., Carelli, R.M., and Wightman, R.M. (2008). Dynamic changes in accumbens dopamine correlate with learning during intracranial self-stimulation. *Proc. Natl. Acad. Sci. USA* 105, 11957–11962.
- Perra, S., Pillolla, G., Melis, M., Muntoni, A.L., Gessa, G.L., and Pistis, M. (2005). Involvement of the endogenous cannabinoid system in the effects of alcohol in the mesolimbic reward circuit: electrophysiological evidence in vivo. *Psychopharmacology (Berl.)* 183, 368–377.
- Phillips, P.E., Stuber, G.D., Heien, M.L., Wightman, R.M., and Carelli, R.M. (2003). Subsecond dopamine release promotes cocaine seeking. *Nature* 422, 614–618.
- Reynolds, J.N., Hyland, B.I., and Wickens, J.R. (2001). A cellular mechanism of reward-related learning. *Nature* 413, 67–70.
- Roitman, M.F., Stuber, G.D., Phillips, P.E., Wightman, R.M., and Carelli, R.M. (2004). Dopamine operates as a subsecond modulator of food seeking. *J. Neurosci.* 24, 1265–1271.
- Ronesi, J., Gerdeman, G.L., and Lovinger, D.M. (2004). Disruption of endocannabinoid release and striatal long-term depression by postsynaptic blockade of endocannabinoid membrane transport. *J. Neurosci.* 24, 1673–1679.
- Scherma, M., Justinova, Z., Zanettini, C., Panlilio, L.V., Mascia, P., Fadda, P., Fratta, W., Makriyannis, A., Vadevel, S.K., Gamaledin, I., et al. (2012). The anandamide transport inhibitor AM404 reduces the rewarding effects of nicotine and nicotine-induced dopamine elevations in the nucleus accumbens shell in rats. *Br. J. Pharmacol.*, in press. Published online May 9, 2011. 10.1111/j.1476-5381.2011.01467.
- Schultz, W., Dayan, P., and Montague, P.R. (1997). A neural substrate of prediction and reward. *Science* 275, 1593–1599.
- Solinas, M., Panlilio, L.V., Antoniou, K., Pappas, L.A., and Goldberg, S.R. (2003). The cannabinoid CB1 antagonist N-piperidyl-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide (SR-141716A) differentially alters the reinforcing effects of heroin under continuous reinforcement, fixed ratio, and progressive ratio schedules of drug self-administration in rats. *J. Pharmacol. Exp. Ther.* 306, 93–102.
- Solinas, M., Goldberg, S.R., and Piomelli, D. (2008). The endocannabinoid system in brain reward processes. *Br. J. Pharmacol.* 154, 369–383.
- Stuber, G.D., Hnasko, T.S., Britt, J.P., Edwards, R.H., and Bonci, A. (2010). Dopaminergic terminals in the nucleus accumbens but not the dorsal striatum corelease glutamate. *J. Neurosci.* 30, 8229–8233.
- Tanimura, A., Yamazaki, M., Hashimoto, Y., Uchigashima, M., Kawata, S., Abe, M., Kita, Y., Hashimoto, K., Shimizu, T., Watanabe, M., et al. (2010). The endocannabinoid 2-arachidonoylglycerol produced by diacylglycerol lipase alpha mediates retrograde suppression of synaptic transmission. *Neuron* 65, 320–327.
- Taylor, K.M., Horvitz, J.C., and Balsam, P.D. (2007). Amphetamine affects the start of responding in the peak interval timing task. *Behav. Processes* 74, 168–175.
- Tecuapetla, F., Patel, J.C., Xenias, H., English, D., Tadros, I., Shah, F., Berlin, J., Deisseroth, K., Rice, M.E., Tepper, J.M., and Koos, T. (2010). Glutamatergic signaling by mesolimbic dopamine neurons in the nucleus accumbens. *J. Neurosci.* 30, 7105–7110.
- van der Stelt, M., Mazzola, C., Esposito, G., Matias, I., Petrosino, S., De Filippis, D., Micale, V., Steardo, L., Drago, F., Iuvone, T., and Di Marzo, V. (2006). Endocannabinoids and beta-amyloid-induced neurotoxicity in vivo: effect of pharmacological elevation of endocannabinoid levels. *Cell Mol. Life Sci.* 63, 1410–1424.
- Ward, S.J., and Dykstra, L.A. (2005). The role of CB1 receptors in sweet versus fat reinforcement: effect of CB1 receptor deletion, CB1 receptor antagonism (SR141716A) and CB1 receptor agonism (CP-55940). *Behav. Pharmacol.* 16, 381–388.

Ward, S., Walker, E., and Dykstra, L. (2007). Effect of cannabinoid CB1 receptor antagonist SR141716A and CB1 receptor knockout on cue-induced reinstatement of Ensure and corn-oil seeking in mice. *Neuropsychopharmacology* 32, 2592–2600.

Wightman, R.M., Heien, M.L., Wassum, K.M., Sombers, L.A., Aragona, B.J., Khan, A.S., Ariansen, J.L., Cheer, J.F., Phillips, P.E., and Carelli, R.M. (2007). Dopamine release is heterogeneous within microenvironments of the rat nucleus accumbens. *Eur. J. Neurosci.* 26, 2046–2054.

Wilson, R.I., and Nicoll, R.A. (2002). Endocannabinoid signaling in the brain. *Science* 296, 678–682.

Xi, Z.X., Spiller, K., Pak, A.C., Gilbert, J., Dillon, C., Li, X., Peng, X.Q., and Gardner, E.L. (2008). Cannabinoid CB1 receptor antagonists attenuate cocaine's rewarding effects: experiments with self-administration and brain-stimulation reward in rats. *Neuropsychopharmacology* 33, 1735–1745.

Yavich, L., and Tiihonen, J. (2000). Patterns of dopamine overflow in mouse nucleus accumbens during intracranial self-stimulation. *Neurosci. Lett.* 293, 41–44.