Uncovering the attenuating effects of cannabinoid receptor blockade on the pursuit of brain stimulation reward.

Ivan Trujillo-Pisanty

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ABSTRACT

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Multiple lines of evidence suggest that blockade of CB1 receptors reduces reward-seeking. However, the reported effects of CB1 blockade on performance for rewarding electrical brain stimulation stand out as an exception. By applying a novel method for conceptualizing and measuring reward seeking, we provide evidence consistent with reward-attenuating effects of cannabinoid receptor blockade: AM-251, a CB1 receptor antagonist, decreases the willingness of rats to pay for medial forebrain bundle stimulation. This analysis clarifies inconsistencies between prior reports, which likely arose from: a) the averaging of data across subjects showing heterogeneous effects and b) the use of methods that cannot distinguish changes in the sensitivity of the reward substrate from changes in reward-substrate gain, reward costs and the value of competing activities such as grooming, resting, and exploring. The results link endocannabinoids to the roles of the latter three factors in reward seeking rather than to the modulation of reward sensitivity.

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PREFACE

Marijuana has been historically one of the most commonly abused drugs. Nonetheless, the scientific study of its interactions with the brain and behavior is relatively new (Iversen, 2000). Understanding marijuana's rewarding effects is particularly relevant as they are sought by millions. We now know that marijuana exerts at least some of its diverse effects by interacting with the endocannabinoid system (Fisar, 2009). This forces us to wonder what the precise role of the endocannabinoid system in reward and motivation is.

The synthesis of the first cannabinoid receptor antagonists was a major breakthrough in the field (Di Marzo, 2009; Iversen, 2000). Their application in behavioral paradigms that evaluate different aspects of reward allowed researchers to demonstrate the importance of the endocannabinoid system in reward (Panagis et al., 2008; Solinas et al., 2007). Despite major advances in the field, the exact role this system plays in complex reward processes has remained obscure (Solinas et al., 2008). Moreover, the existence of contradictory findings has made it impossible to reach a general agreement. The observation of unforeseen depressive side effects in clinical trials that assessed the use of cannabinoid antagonist for weight loss (Moreira and Crippa, 2009) also suggests that we have not fully understood their role.

We suggest that the present work advances the effort to delineate a functional role for endocannabinoids in reward seeking by the application of a new model and novel

methodology evolved from it. This novel approach has also helped us in understanding why some of the previous studies seem contradictory.

The current study was recently submitted for publication in a peer-reviewed journal. The attached document is a version of the submitted manuscript modified to conform with Concordia University's thesis format. This work is the result of the combined effort from the following coauthors:

Ivan Trujillo-Pisanty: Conducted the experiments, analyzed the data and suggested simulating the 2D analysis. He wrote the first draft of the article and continued correcting and revising it until its submission. He collaborated with Kent Conover in the creation of two movies that illustrate the ambiguity of 2D representations and which were submitted as supplementary material.

Giovanni Hernandez: Showed Ivan how to conduct the experiments and contributed with the surgery and training of one of the subjects. He made suggestions on stimulation adjustments during the training of some rats. He also revised and made corrections in preparing the article for submission.

Kent Conover: Programmed all MATLAB procedures for fitting, comparing and graphing. He also gave valuable advice in fitting and analyzing the data. Along with Peter Shizgal, he suggested resampling by survey to improve the quality of our estimates. He

played a major role in creating two movies that were submitted as supplementary material.

Joseph Cheer: Suggested exploring the effects of cannabinoid antagonists with the mountain model. He revised and corrected the later versions of the manuscript for submission.

Peter Shizgal: Created the mountain model and adapted it to include the conditioned reward parameter. He supervised each step of the experiment closely and made suggestions during the training and data acquisition phases. He developed the 2D simulation. Along with Kent Conover he suggested resampling by survey to improve our estimates. He played a major role in revising and correcting the article until its submission. All experiments were conducted using the facilities and resources under his supervision.

Uncovering the attenuating effects of cannabinoid receptor blockade on the pursuit of brain stimulation reward.

Ivan Trujillo-Pisanty, Giovanni Hernandez, Kent Conover, Joseph F. Cheer and Peter Shizgal. Submitted

It has been suggested that signaling mediated by the cannabinoid 1 receptor (CB1R) modulates the behavioral impact of rewards. Rodents pretreated with the CB1R antagonists, rimonabant or AM-251, show decreased willingness-to-pay for food reward in progressive-ratio tests (Rasmussen and Huskinson, 2008) and blunt appetitive responses to palatable foods in the taste-reactivity test (Jarrett et al., 2007). Conversely, CB1 receptor agonists increase operant responding for food reward (Solinas and Goldberg, 2005) and produce a conditioned place preference (Valjent and Maldonado, 2000). Moreover, CB1R blockade impairs the conditioned place preferences produced by diverse rewards, such as nicotine (Forget et al., 2005), stimulants (Yu et al., 2009) and morphine (Singh et al., 2004). Likewise, self-administration of various drugs of abuse is reduced by CB1R blockade (Filip et al., 2006; Shoaib, 2008; Xi et al., 2008). Dopamine (DA) release in the nucleus accumbens (NAc) has been implicated in reward and motivation (Wise, 2008). Mice lacking CB1Rs show decreased DA release in the NAc in response to morphine (Mascia et al., 1999), cocaine (Li et al., 2009) and ethanol administration (Hungund et al., 2003). Pharmacological blockade of CB1R inhibits NAc dopamine release in response to various drugs of abuse (Cheer et al., 2007) whereas pharmacological activation of CB1Rs enhances DA release in this region (Cheer et al., 2004; Solinas et al., 2006).

Many vertebrates will work vigorously for electrical stimulation of the medial forebrain bundle (Vetulani, 2001). This phenomenon, known as intracranial self-stimulation (ICSS), has seen long service in the study of the neurochemical basis of reward. The effect that leads the rat to seek out the electrical stimulation is called "brain stimulation reward" (BSR). It has been shown that BSR can compete with, summate with, and substitute for natural rewards (Conover and Shizgal, 1994; Conover et al., 1994; Green and Rachlin, 1991). Thus, the neural signal responsible for BSR appears to be related closely to those that encode the value of natural goal objects.

The curve-shift method is regarded as the premier method for quantitative assessment of the effects of drugs on BSR (Edmonds and Gallistel, 1974, 1977; Miliaressis et al., 1986). Psychometric curves are obtained that map the strength of the rewarding stimulation (e.g., the pulse frequency or current) into the vigor of instrumental performance (e.g., the rate of lever pressing or running speed in an alley). The effects of pharmacological manipulations are quantified in terms of drug-induced displacement of the psychometric curve along the axis representing stimulation strength.

Inconsistent results have been obtained when the curve-shift paradigm has been used to assess the effects of CB1R ligands. Some researchers have found that CB1R blockade decreases the effectiveness of the electrical reward, i.e., the drug shifts the psychometric curve to the right (De Vry et al., 2004; Deroche-Gamonet et al., 2001). However, others have failed to find such effect (Vlachou et al., 2003, 2005; Xi et al.,

2008) or have reported it only following administration of very high doses of rimonabant (Arnold et al., 2001; Xi et al., 2008). This inconsistency stands in sharp contrast to the reported effects of CB1R ligands on performance for food and drugs, effects about which there is consensus (Solinas et al., 2008).

In this report, we address cannabinoid receptor modulation of BSR from a new perspective (Arvanitogiannis and Shizgal, 2008; Hernandez et al., submitted). Within a quantitative framework that incorporates reward costs, we distinguish between changes induced by CB1R blockade in the sensitivity and gain of brain reward circuitry. Changes in sensitivity alter the stimulation strength required to produce a half-maximal reward whereas changes in gain alter the maximal reward attainable. Whereas sensitivity is analogous to the K_M of an enzyme, gain is analogous to the v_{max}. (Heyman, 1988; Heyman and Beer, 1987).

Pursuit of rewards in operant paradigms depends on their intensity and cost, as well as on the value of activities, such as grooming, resting, and exploring, that compete with the reward supplied by the experimenter (Herrnstein, 1970; Herrnstein, 1974; Heyman, 1988; Killeen, 1972). The approach adopted here is based on the quantitative account provided by Arvanitogiannis and Shizgal (2008), which links the pursuit of rewarding brain stimulation to reward intensity, reward cost and the value of competing activities (Figure 1A). In an updated version of the measurement method derived from their model (Hernandez et al., submitted), the experimenter varies both stimulation

strength, as determined by the pulse frequency, and the opportunity cost of BSR, as determined by the time required to harvest a stimulation train ("price"). As illustrated by

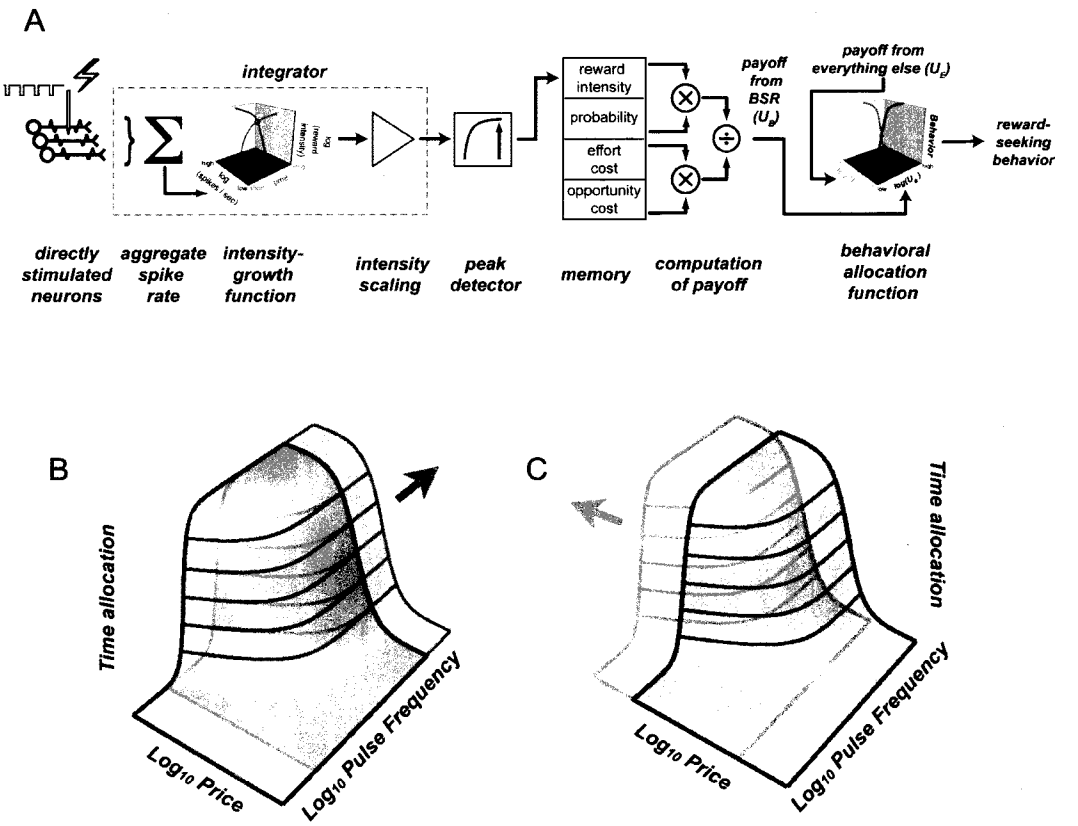

Figure 1. Graphical representation of the mountain model.
(A) In the initial stages of processing an intensity growth function transforms the aggregate spike rate induced by the stimulation train in the directly stimulated neurons into a reward-intensity signal. Following rescaling, the peak reward-intensity is transferred to memory. The payoff from BSR (U_B) is computed by discounting the stored reward-intensity value by the probability that a reward will be delivered when the work requirement has been met and by the effort and opportunity cost of the reward. The proportion of time the animal invests in pursuit of BSR is determined by a comparison of U_B, suitably transformed (Hernandez et al., submitted), and the sum of the suitably transformed values of both BSR and the payoff from competing activities (U_E). **(B)** Changes in the location parameter of the intensity-growth function (F_{hm}) produce shifts along the frequency axis of the 3D space. **(C)** Variations in the scaling of the output of the intensity-growth function, in the probability or cost of reward, or in the value of competing activities produce shifts along the price axis of the mountain. (See also Equation 1 in Appendix).

the three-dimensional (3D) graphs in Figures 1B and 1C, the proportion of a subject's time (time allocation: TA) devoted to seeking out the stimulation increases as a function of pulse frequency and decreases as a function of price. The 3D structure represented by the colored surfaces (which were produced by Equation 1 in Appendix) is dubbed the "reward mountain."

The mountain is shifted along the pulse-frequency axis by drug-induced changes in the sensitivity of the neural circuitry underlying BSR (Figure 1B). In contrast, the mountain is shifted along the price axis by changes in gain, in perceived costs, or in the value of competing activities (Figure 1C). These orthogonal movements are reflected in the location parameters of the model: the pulse frequency required to produce a half-maximal reward, F_{hm}, determines the position of the mountain along the pulse-frequency axis whereas the price at which the rat will spend half its time working for a maximal BSR, P_e, determines the position of the mountain along the price axis. As shown in Figure 1A, these two parameters reflect different stages in the process that translates the volley of action potentials triggered by the stimulation into reward-seeking behavior. Drug-induced changes in F_{hm} (Figure 1B) reflect drug action prior to the output of the ("intensity-growth") function that translates the rate of firing in the directly activated neurons into a subjective reward intensity (Gallistel and Leon, 1991; Leon and Gallistel, 1992; Simmons and Gallistel, 1994). In contrast, the P_e parameter reflects stages of processing beyond the output of intensity-growth function (Figures 1A and 1C) (Arvanitogiannis and Shizgal, 2008; Hernandez et al., submitted).

In traditional ICSS studies, the 3D structure is projected implicitly onto a two-dimensional (2D) plane, defined, in the curve-shift paradigm, by a measure of performance (e.g., response rate) and a measure of stimulation strength (typically pulse frequency or current). The resulting curves are silhouettes of the 3D structure. Drug effects are quantified in terms of displacements of these silhouettes. However, such displacements are inherently ambiguous (Arvanitogiannis and Shizgal, 2008; Hernandez et al., submitted): A given shift of a 2D silhouette can be produced by drug action at different stages of neural processing, as reflected in the two location parameters of the 3D structure. Thus, in the traditional 2D representations, such as curve-shift or progressive-ratio measurements, changes in the sensitivity of the BSR substrate (indexed by F_{hm}) cannot be distinguished from changes in the gain of the BSR substrate, in subjective costs, or in the value of competing activities (indexed collectively by P_e). However, mountain-based 3D measurements distinguish these different effects unambiguously.

To date, all studies of the effects of cannabinoids on ICSS have used 2D analysis; we show below that this could be partly responsible for the inconsistency between reported results. The common practice of averaging data across subjects could also have contributed, as demonstrated below. The present study is the first to characterize the effect of CB1R blockade on the pursuit of BSR by means of a model-based approach entailing 3D measurement and statistical methods appropriate for analysis of single-subject data. This novel methodology yields a consistent effect of CB1R blockade: decreased willingness to pay for rewarding electrical stimulation in the absence of any systematic change in the sensitivity of the neural circuitry responsible for BSR.

EXPERIMENTAL PROCEDURES

Subjects

Subjects were thirteen male Long-Evans rats from Charles River Breeding Farms (St. Constant, Quebec). The rats were housed in Plexiglas® cages in a vivarium with controlled temperature and reversed 12-12h dark/light cycle. Food and water were available *ad libitum*. The behavioral procedures were conducted during the dark phase of the cycle, between 7:30 am and 2:00 pm. All procedures complied with the principles of the Canadian Council of Animal Care.

Electrode implantation

Rats weighed 400-550g at the time of surgery. We administered atropine sulfate (0.05 mg/kg s.c) to reduce bronquial secretions. Anesthesia was induced with Ketamine-Xylazine (10-100mg/kg i.p) and maintained with isoflurane vapor. Penicillin (0.3ml/kg i.m) was injected to prevent infections. Prior to mounting the rat in the stereotaxic frame, Xylocaine jelly was applied to the external auditory meatus to reduce discomfort from the ear bars. Monopolar stainless-steel electrodes were constructed from 000 insect pins and insulated with formvar to within 0.5mm of the tip. The electrodes were bilaterally aimed at the MFB at the level of the lateral hypothalamus (LH) (AP:-2.8, ML:+/- 1.7, DV: 8.7-8.9 from the skull). Four stainless-steel jeweler screws were threaded into pilot holes drilled in the skull; the electrodes were anchored to these screws with dental acrylic. A length of wire wrapped around two of the screws served as the current return. Gold-plated Amphenol connectors, attached, via a short length of wire, to each of the electrodes and the skull-screw return, were inserted into a McIntyre Miniature Connector

(Scientific Technology Centre, Carleton University, Ottawa, Ontario, Canada), which was attached to the skull screws with dental acrylic to form a head cap. Buprenorphine (0.05 mg/kg, s.c) was administered immediately following surgery to reduce subsequent pain. Rats were allowed 5-7 days of recovery before behavioral training began.

Apparatus

We used four plastic operant boxes (30 x 21 x 51 cm) with a mesh floor and a clear Plexiglas® front. Each box was equipped with a flashing light, located 10cm above the floor mesh, and a retractable lever (ENV–112B, MED Associates, St. Albans, Vermont) mounted on the right side wall. A 1 cm light was located 2 cm above the lever and was activated when the rat depressed the lever.

The temporal parameters of the electrical stimulation were set by a computer-controlled digital pulse generator, and pulse amplitude was determined by a computer-controlled constant-current amplifier. Stimulation consisted of 0.5 s trains of cathodal pulses, 0.1 ms in duration. The stimulation current was routed to the rat through a multi-channel slip ring that allowed the rat to circle without tangling the leads. Experimental control and data acquisition were handled by a personal computer running a custom-written program ("PREF") developed by Steve Cabilio. The stimulation was monitored on an oscilloscope by displaying the potential drop across a 1% precision resistor (1KΩ) in series with the rat.

Behavioral task training

For each rat, we determined the stimulating electrode and the current-frequency combination that supported vigorous lever pressing with minimum aversive side effects. From that point onwards the current and stimulating electrode were held constant. Rats were then trained to keep the lever depressed for a cumulative time of four seconds in order to receive the stimulation. Once this task had been mastered, training commenced on the "frequency-sweep" procedure. Each sweep consisted of a set of trials during which the stimulation parameters were held constant, and the rat had the opportunity to harvest as many as 20 rewards. Following delivery of each reward, the lever was disarmed and retracted for 2 or 3 s. The pulse frequency during the first three trials was set to the highest value the rat could tolerate without signs of aversion or forced movement. Over the subsequent eight trials, the pulse frequency was decreased systematically from trial to trial in equal proportional steps. The dependent variable was a corrected measure of the proportion of trial time that the lever was depressed ("time allocation"), as explained in detail by Breton et al. (2009). The range of pulse frequencies was selected to drive time allocation from its maximal to its minimal values, in sigmoidal fashion. Every trial was preceded by a ten second inter-trial interval signaled by a flashing light. During the last two seconds of this period rats received priming stimulation consisting of two stimulation trains at the maximum pulse frequency that the rat could tolerate, delivered at 1 train s^{-1}.

After the subject showed consistent high asymptotic values of time allocation (not lower than 0.8) in at least the first two trials and low asymptotic values (below 0.2) in at least the last two trials of each determination, we changed the work requirements of three

of the determinations. The rats had to hold down the lever for increasing cumulative periods (i.e. prices) to obtain a stimulation train of maximal strength. We refer to these determinations as "price sweeps" because the opportunity cost is systematically increased under such circumstances while the stimulation strength is held constant. The duration of each trial was adjusted to allow the rat to harvest a maximum of 20 rewards. After consistently high and low asymptotic time-allocation values (≥ 0.8 and ≤ 0.2, respectively) were observed in price-sweep data, a new "radial" sweep was added, replacing one frequency sweep and one price sweep. In a radial sweep, the required price increased, and the stimulation strength decreased, simultaneously across sequential trials. The stimulation-price combinations and the spacing between the trials were calculated so that the vector described by the radial sweep in the parameter space ($\log_{10}(P)$ versus $\log_{10}(F)$) passed through, or very near, the point defined by the fitted values of the location parameters ($\log_{10}(P_e)$, $\log_{10}(F_{hm})$) (see Figures 3A and 3B). This was achieved using the data from the frequency and price sweeps and a simulator developed by Yannick Breton and implemented in MATLAB (The Mathworks, Natick, MA). Two sweeps of each type were run during every session. We use the term "survey" to refer to the combination of a frequency, a price and a radial sweep; these provide the minimal dataset required to fit the mountain model. The sequence of sweeps was random within session for subjects C8-C14 and random within survey for C17-C20. In the latter case, the rats had to complete a full survey before any of the sweeps were repeated; this adjustment was made in order to increase the power of the resampling based surface-fitting approach (see below). Each rat performed under these conditions for four sessions, and then the model was fitted (see "Model fitting and comparisons" section). If the radial sweep deviated excessively from

the fitted values of ($\log_{10}(P_e)$, $\log_{10}(F_{hm})$) or if the upper or lower asymptotic time-allocation values were insufficiently well defined, the sequence of prices and pulse frequencies was readjusted. Each rat was considered ready for drug testing when its responding was consistent throughout sessions and the trajectory of the radial sweep passed sufficiently close to ($\log_{10}(P_e)$, $\log_{10}(F_{hm})$). Rats required five weeks of training, on average, to reach the drug-testing phase. Rats that failed to meet the inclusion criteria described above were excluded from the experiment.

Testing under the influence of AM-251 and its vehicle

Each session consisted of a warm-up frequency sweep, followed by two price, two radial and two frequency sweeps, either randomized within sessions (rats C8-C14) or randomized within surveys (rats C17-C20).

AM-251 (3 mg/kg; Tocris Bioscience) was diluted in a 1:1:18 solution of ethanol, cremophor and physiological saline. The drug or its vehicle were administered at a volume of 3 ml/kg, i.p., thirty minutes before each behavioral test. This dose was chosen in accordance to previous studies (Arnold et al., 2001; De Vry et al., 2004; Deroche-Gamonet et al., 2001; Vlachou et al., 2003, 2005; Xi et al., 2008). The stimulation frequencies and prices in the vehicle sessions were the same as those determined in the training phase. During the drug sessions, the price values tested in the price sweep were decreased by 0.1-0.2 $\log_{10}$ units on the basis of leftwards shifts along the price axis observed during pilot testing (data not shown). At least one washout day followed each drug session to allow for elimination of the drug. Rats received vehicle injections on

Mondays and Thursdays, drug injections on Tuesdays and Fridays; Wednesdays, Saturdays and Sundays were washout days. Eight to 12 test sessions, 5-6 h in duration, were run in both the drug and vehicle conditions. Roughly three months were required, following the initial surgery, to complete testing each subject.

Model fitting and comparisons

Among the objectives of the model-fitting approach were unbiased estimates of location-parameter (F_{hm}, P_e) values and their dispersions for each subject. This was accomplished by means of a MATLAB (The Mathworks, Natick, MA) procedure developed by Kent Conover, based on the non-linear least-squares routine in the MATLAB Optimization Toolbox and resampling methods (Efron and Tibshirani, 1994). A primary fit of the six-parameter model presented in Eq. 1 was performed independently to the data from each session (subject C8-C14) or survey (C17-C20) in each condition, this was done using the "location-specific approach" (Hernandez et al., submitted). This approach entails fitting individual values of the two location parameters to the data for each session or survey while using common values of the four remaining parameters. The reason for this procedure is to protect the values of the two slope parameters, a and g (see Equation 1 in Appendix), from the degradation that would ensue from fitting common values of all parameters to datasets that shift in the parameter space from session to session (Hernandez et al., submitted); such shifts would be expected to arise from unavoidable variation in drug administration, absorption, etc. Following the primary fit, the data were then resampled with replacement by session or survey, 1000 times; the model was fitted to each resampled dataset as described above. Estimates of the mean

value of each parameter and the corresponding 95% confidence interval were computed over the 1000 fits; in the case of the location parameters, the session-specific values were averaged within each set of fits to a given resampled dataset. The 95% confidence intervals were percentile-based: they exclude the lowest and highest 25 of the 1000 values (Figure 3).

The seven-parameter model described elsewhere (Hernandez, et al., submitted) allowed us to account for the exceptionally high time allocation observed at the lower pulse frequencies during the frequency sweeps for subject C17; according to the Akaike information criterion (Efron and Tibshirani, 1994), this model provided a better fit (data not shown) than the standard model, but only in the case of this one rat.

A difference vector was constructed for each location parameter in each subject by subtracting, element by element, the 1000 estimates for the AM-251 condition from the 1000 estimates for the vehicle condition. The mean shifts reported here represent the mean of this difference vector whereas the 95% confidence intervals are simply its 2.5^{th} and 97.5^{th} percentiles (Figure 5). If the confidence interval did not include zero, the difference between conditions was considered statistically reliable, with an alpha level of 0.05.

Simulation of 2D curve-shifts

On the basis of the mountain model and the fitted parameter values for each rat, we estimated the F_{m50} values that would have been obtained in a conventional curve-shift

experiment corresponding to our own subjects (see Equation 2 in the Appendix). To account for the low price paid for reward when the commonly used, continuous-reinforcement schedule is in force, we set the price to 0.1 s. In accord with the practice in most prior studies liking CB1Rs with BSR (Arnold et al., 2001; De Vry et al., 2004; Deroche-Gamonet et al., 2001; Vlachou et al., 2003, 2005; Xi et al., 2008), we compared the simulated F_{m50} values for the drug and vehicle conditions using a paired-sample t-test.

Prussian Blue technique, Formal thionine staining and histology

Rats were overdosed with Ketamine-Xylazine. A 1-mA anodal current was passed through the electrode that was used to deliver the stimulation during the behavioral testing for 15 seconds. Rats were then perfused intracardially with physiological saline followed by a Prussian blue solution (10% formalin, 3% potassium ferricyanide, 3% potassium ferrocyanide and 0.5% trichloroacetic acid). The brains were extracted and kept in a 10% formalin solution for at least 7 days and then transferred to a 30% sucrose-formalin solution for three days. The brains were sliced in 40 μm coronal sections using a cryostat and mounted on precoated microscope glass slides (Fisherbrand Superfrost/plus, Fisher Scientific, USA). The slides were submerged in acid acetone (20% glacial acetic acid, 80% acetone) for five minutes, followed by two minutes in distilled water. A formal thionine solution (1% thionine in 10% formalin) at 60-80° C was applied for 6-7 min, followed by a 1-min distilled-water rinse. The slices were then submerged in acid alcohol (5% glacial acetic acid, 45% distilled water, 50% isopropyl alcohol) for sixty s, followed by a five min in isopropyl alcohol and ten min in Histochoice (Amresco, USA). Electrode

tips locations were determined under low magnification and reference to the rat-brain atlas of Paxinos and Watson (Paxinos and Watson, 2007).

RESULTS AND DISCUSSION

A reward was delivered when the cumulative time that the rat held down a retractable lever reached a criterion value (the opportunity cost or "price" of the stimulation). The reward consisted of a train of current pulses delivered to the medial forebrain bundle (MFB) at the level of the LH. All of the electrode tips fell in this region (Figure 2).

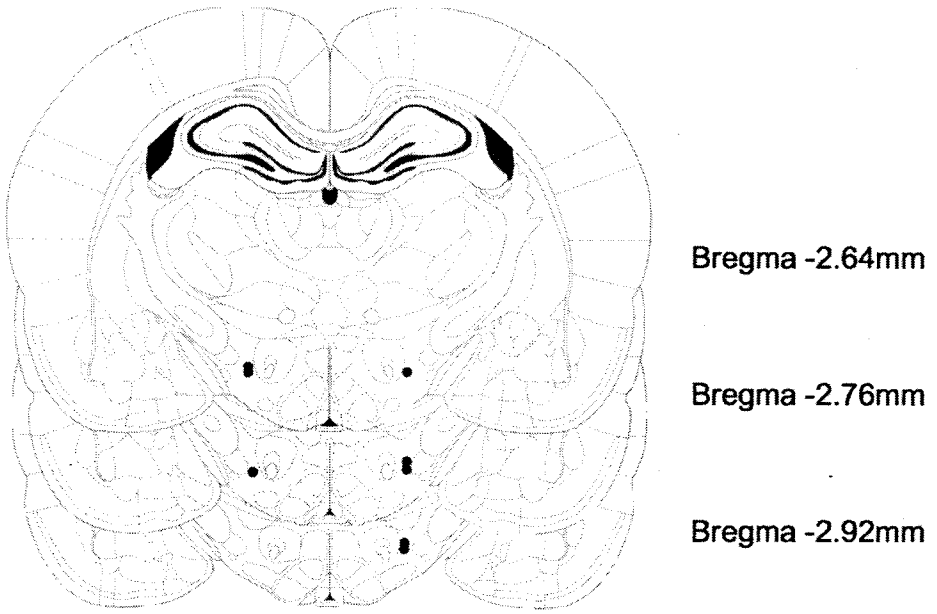

Figure 2. Electrode placement.
The location of each electrode tip is shown. All placements fell within the boundaries of the MFB, at the level of the LH, as determined using the Atlas by Paxinos and Watson (2007).

We measured the proportion of trial time that the lever was depressed as a function of the pulse frequency and the price. We fitted our model to determine the F_{hm} and P_e parameters values for each rat following the administration of the CB1 receptor blocker, AM-251, or its vehicle. An example of the data from a single subject is shown in figures 3 and 4.

Changes in the values of the location parameters across conditions were assessed independently for each rat (Figure 5). Shifts in the value of the F_{hm} parameter met the criterion for statistical reliability in the data from three of eight rats and ranged from -.119 to .194 common logarithmic units. However, the direction of these shifts was inconsistent; in the case of Rat C8, F_{hm} decreased in the drug condition whereas in the cases of Rats C11 and C14, it increased (Figure 6). In contrast, we found a reliable decrease in the value of P_e following drug administration in seven of the eight rats. Figure 6 shows that the size of these shifts ranged from -0.084 to -0.242 common units (17.6 – 42.7% decreases in P_e). These results suggest that the primary effect of CB1 receptor blockade on reward pursuit arose from an action of the drug beyond the output of the intensity-growth function (Figure 1). Such actions could include downward rescaling of integrator output (i.e., a decrease in gain), or increases in subjective opportunity costs (subjective valuation of the time entailed to earn a reward), subjective efforts costs (subjective valuation of the physical work entailed in earning a reward), and the value of competing activities. (Reward was delivered every time the work requirement was satisfied and hence, drug-induced changes in subjective probability were

unlikely.) Thus, CB1Rs play their principal role beyond the neural circuitry that determines reward sensitivity.

CB1 receptors are the target of at least two endogenous ligands: anandamide and 2-arachidonoylglycerol. It has been suggested that these two lipids play different behavioral roles (Long et al., 2009). Given that blockade of the CB1R interferes with the binding of both endocannabinoids, there is no way, at present, to partition the observed effects between them. This might be achieved through the use of novel pharmacological tools that selectively and differentially prevent the degradation of these compounds (Fegley et al., 2005; King et al., 2007).

Our results show that preventing CB1R activation diminishes pursuit of BSR. This effect may reflect an interaction of CB1R blockade with other neurotransmitter systems implicated in reward pursuit. We have recently shown that increasing dopamine tone in the NAc causes rightward shifts along the price axis (Hernandez et al., submitted), an effect opposite in sign to the one reported here. Interestingly, it has also been shown that cannabinoid antagonists diminish dopaminergic activity in areas involved in drug-induced reward seeking (Cheer et al., 2007). Dopamine tone in the NAc increases during MFB self-stimulation in a manner that appears to promote pursuit of the electrical reward (Hernandez et al., 2007; Hernandez et al., 2006; Hernandez and Shizgal, 2009). Thus, AM-251-induced decreases in willingness to pay for BSR may arise by counteracting stimulation of DA tone in the ventral striatum.

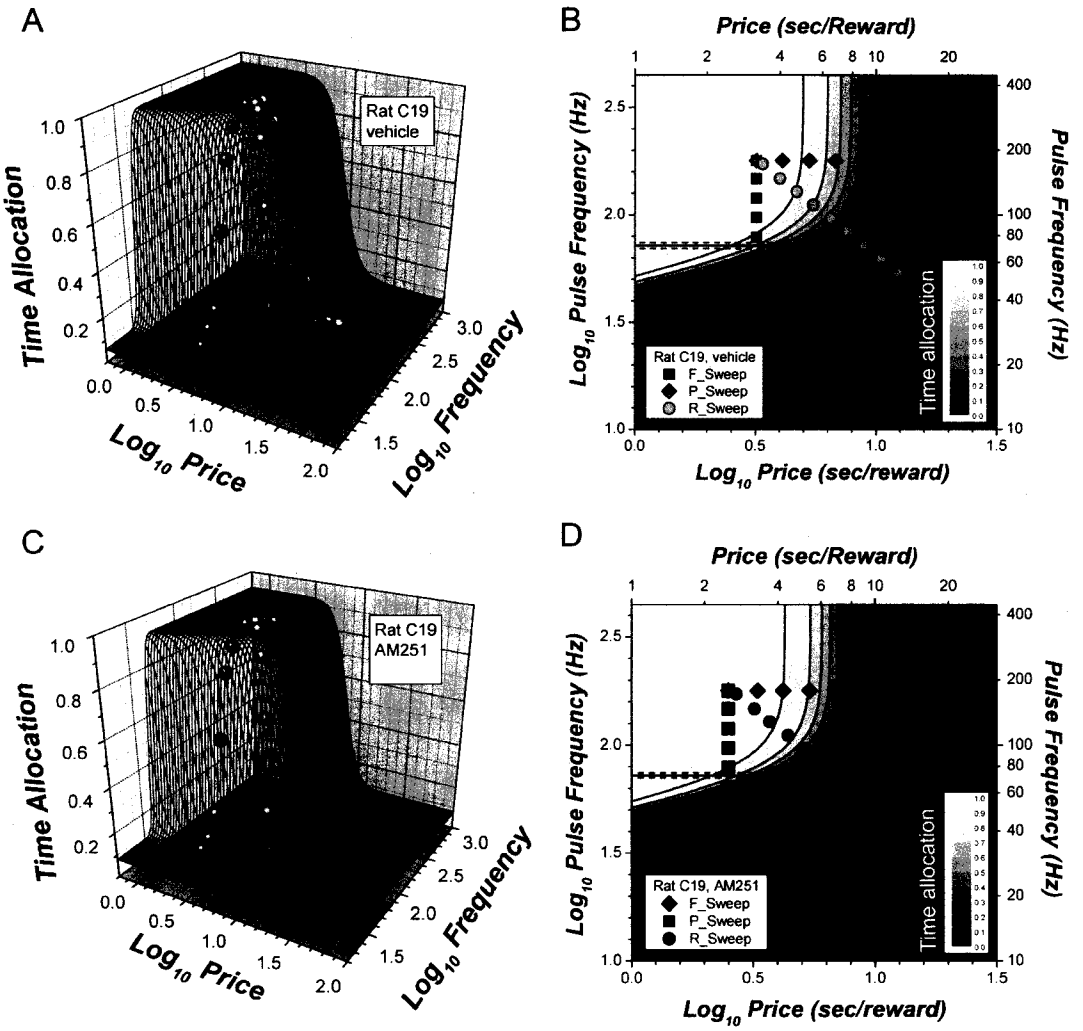

Figure 3. Fit of the mountain model to the time-allocation data obtained following treatment with AM-251 and vehicle.

(A) The surface fitted to the vehicle data from one subject (Rat C19) is shown as a wire mesh. The red, green and blue dots represent mean time-allocation values for the frequency, radial and price sweeps respectively. The solid vertical red and blue lines represent the fitted values of the location parameters, F_{hm} and P_e, respectively. **(B)** Contour graph corresponding to panel A. The solid vertical red and blue lines again represent the values of the location parameters where the accompanying dashed lines represent the corresponding upper and lower 95% low confidence intervals. **(C)** The surface fitted to the AM-251 data from subject C19 is shown along with red, green and blue dots representing mean time-allocation values for the frequency, radial and price sweeps respectively. **(D)** Contour graph corresponding to panel C.

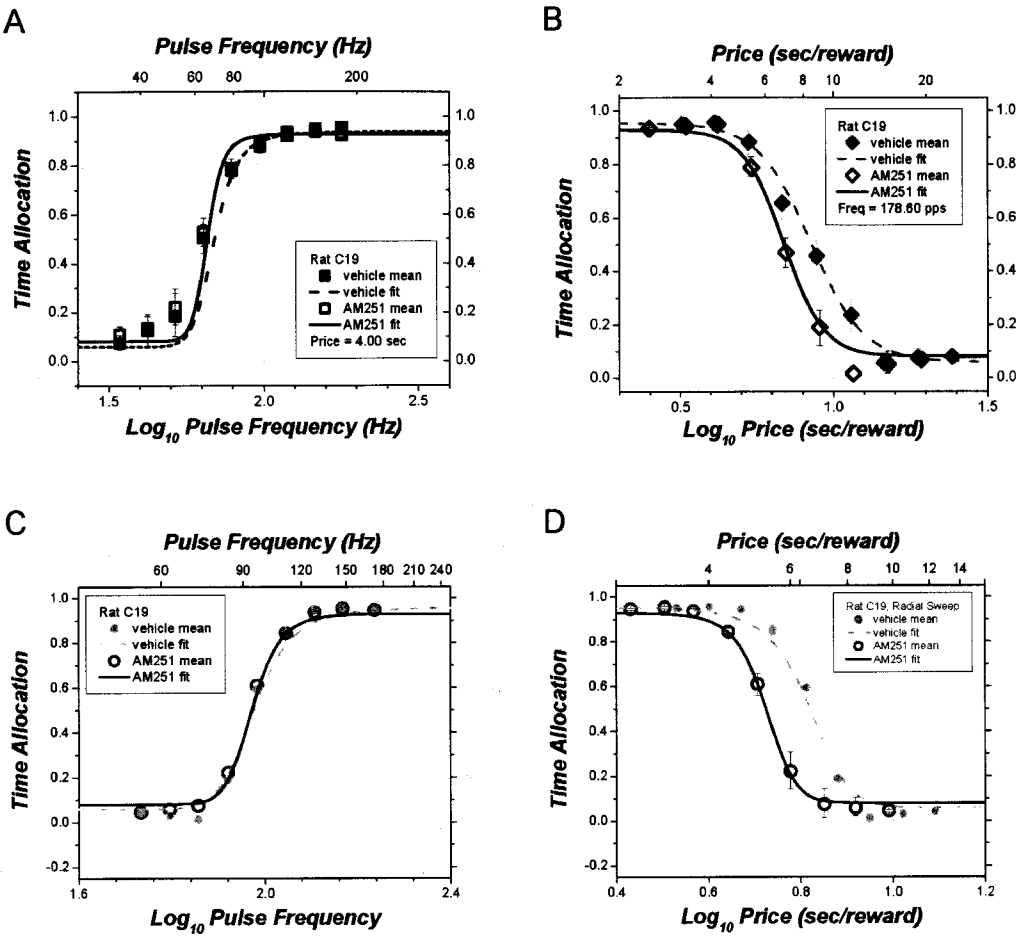

Figure 4. Two-dimensional representations of results from Rat C19.
(A) The frequency-sweep data for each condition along with the 2D projections of the fitted surfaces. **(B)** The price-sweep data for each condition along with the 2D projections of the fitted surfaces. **(C)** The radial-sweep data and corresponding 2D projections, shown against the pulse- frequency axis. **(D)** The radial-sweep data and corresponding 2D projections, shown against the price axis.

The results reported here illustrate an important methodological point: restricting the collection and analysis of ICSS data to two dimensions and averaging results across subjects can obscure effects that are discernable clearly when performance is measured as a function of both the strength and cost of BSR and in a manner that supports single-subject analysis. This point is illustrated by deriving from our data a measure analogous

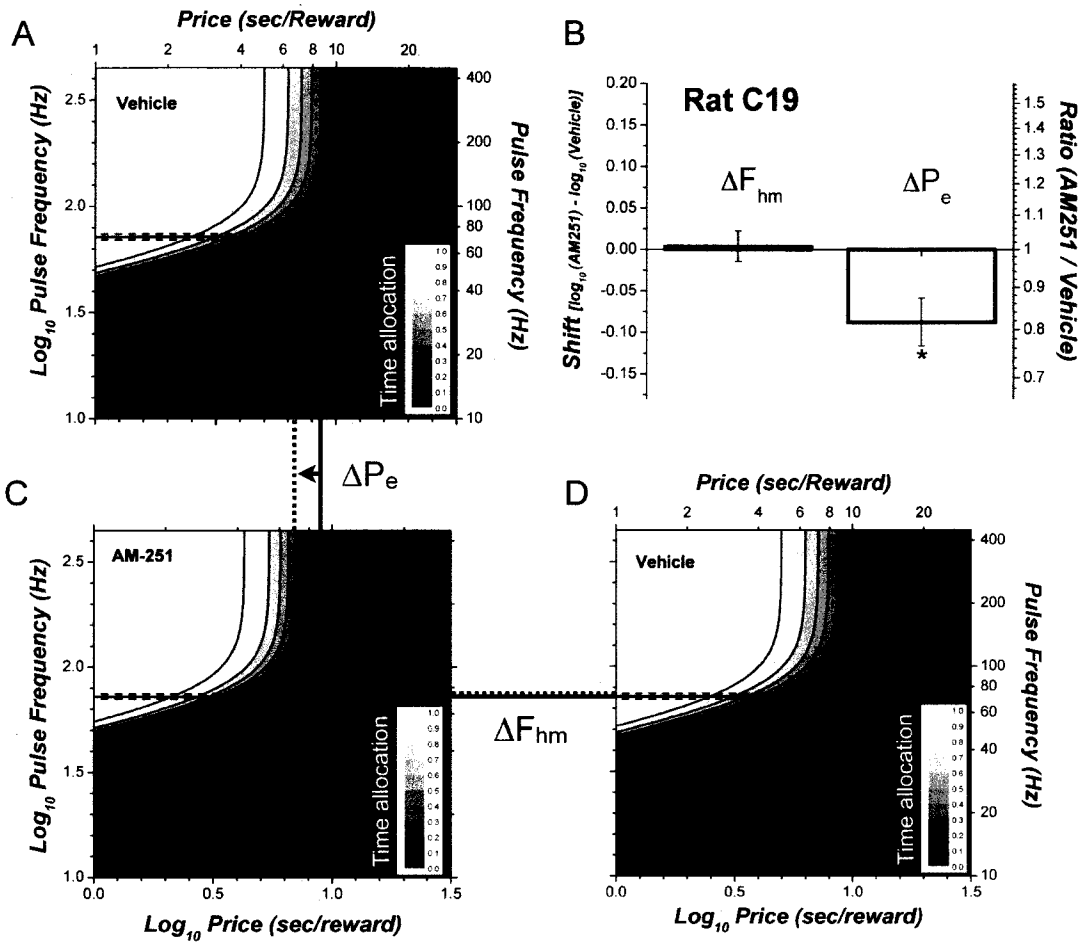

Figure 5. AM-251-induced shifts in the location of the mountain.
(A and D) The contour graph of the surface fitted to the vehicle data (Figure 3, Rat C19) is shown twice. This representation provides reference points for visualizing any AM-251-induced shifts of the mountain along the price and pulse-frequency axes. **(C)** The contour graph of the surface fitted to the data obtained from the same subject in the drug condition. The solid red and dashed red horizontal lines represent the values of the F_{hm} parameter for the vehicle or AM-251 condition, respectively. Note the near overlap of these two lines. The solid blue and dashed blue vertical lines represent the values of the P_e parameter for the vehicle or drug condition, respectively. The accompanying blue arrow indicates a statistically reliable decrease in P_e. **(B)** The shifts in both location parameters are contrasted in the bar graph; these shifts are the difference between the common logarithmic value of each parameter for the drug and vehicle conditions. Error bars denote 95% confidence intervals. In this subject, AM-251 shifted the mountain along the price, but not the pulse-frequency axis.

to the 2D group curve-shifts that have typically been measured. We used the mountain model to derive a widely used location parameter for psychometric curves obtained in curve-shift experiments, F_{m50}, the pulse frequency that supports a half-maximal level of performance (Figure 7A and 7B). In concert with conventional practice, we compared the F_{m50} estimates for the drug and vehicle conditions by means of a paired sample t-test (Arnold et al., 2001; De Vry et al., 2004; Deroche-Gamonet et al., 2001; Vlachou et al., 2003, 2005; Xi et al., 2008). Despite the reliable shifts along the price axis in 7/8 rats, the difference in F_{m50} values failed to cross the statistical threshold [t(7)= 1.885, p> 0.05] (Figure 7C). This shows that the 3D methodology permits the detection of differences

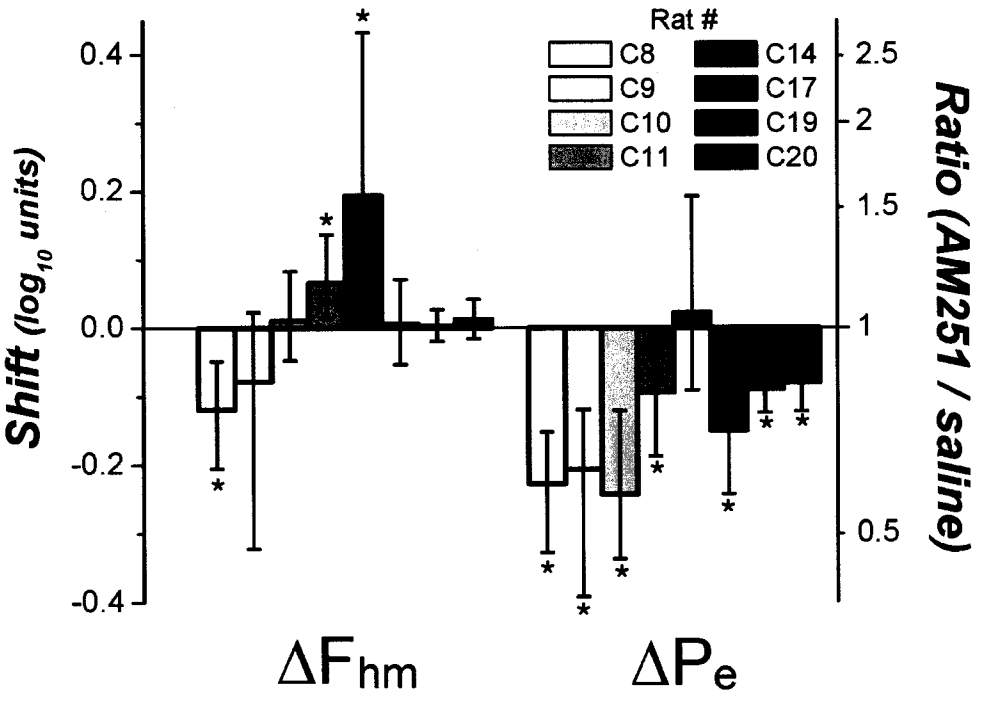

Figure 6. AM-251-induced shifts in the location parameters for all subjects. Drug-induced changes in F_{hm} are shown on the left and those in P_e on the right. Error bars denote 95% confidence intervals. Note that F_{hm} did not change in most cases and that the directions of the three statistically reliable shifts are inconsistent. In contrast, a consistent decrease in P_e was found in seven of eight subjects.

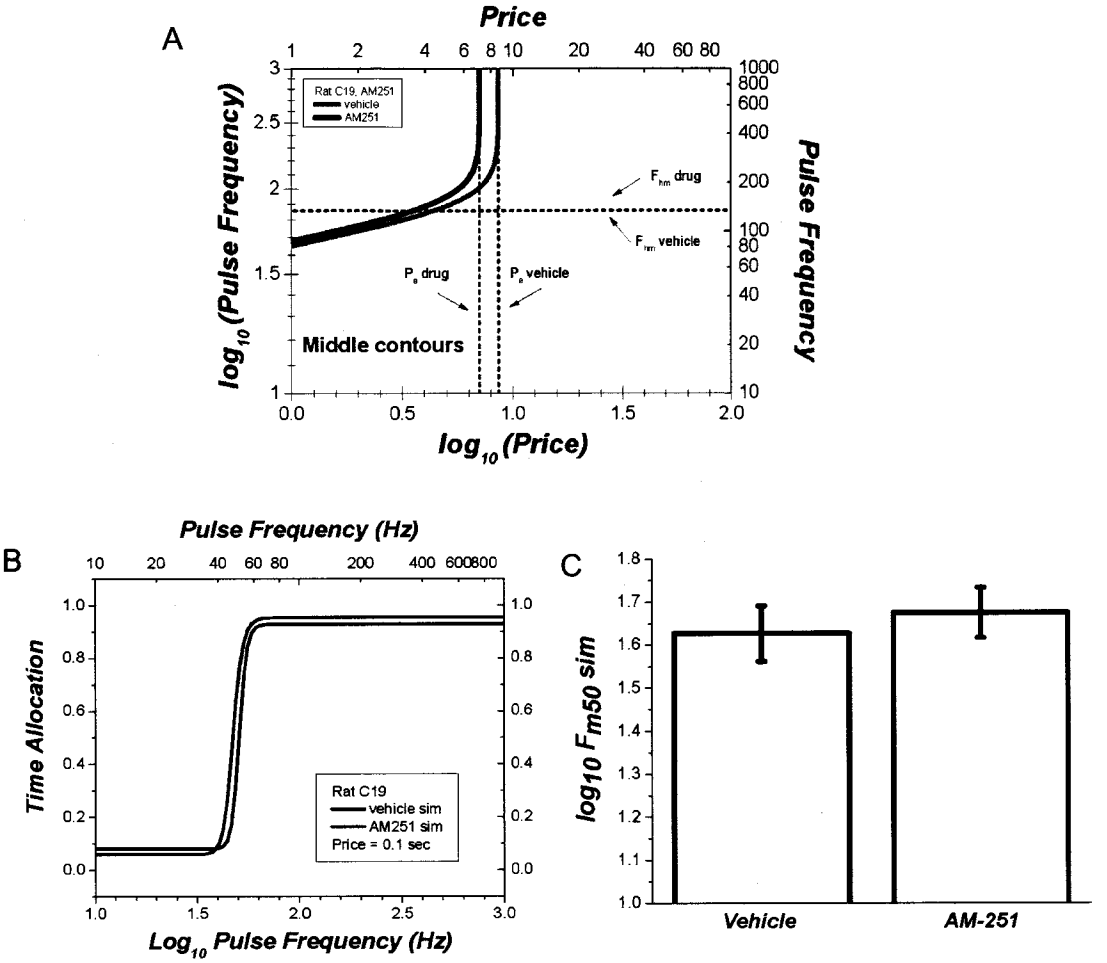

Figure 7. Derivation of the equivalent 2D curve-shift following CB1 receptor blockade.

(A) The contour lines mid-way between TA_{max} and TA_{min}. The data are drawn from the surfaces fitted to the data from Rat C19. Dashed lines represent the values of the location parameters. Note the leftward shift in P_e values caused by the drug, and the near-absence of such a shift along the pulse-frequency axis. Due to the gentle slope of the diagonal portion of the contour line, a substantial displacement along the price axis is translated into a much smaller shift along the pulse-frequency axis. **(B)** Simulated curves showing how the frequency-sweep data from subject C19 would appear had they been obtained using the standard curve-shift method. Such a small shift would almost certainly be lost in the noise. Values of the pulse frequency corresponding to half-maximal behavioral allocation (*Fm50* values) were derived from the mountain model and the fitted parameter values; these were then averaged across subjects. The resulting means and standard errors are shown in panel. **(C)** The results of a paired-sample t-test do not meet the 0.05 criterion for statistical reliability. Thus, the effect of AM-251, so clear in the contour-line representation in panel A, cannot be discerned using the conventional method. (See also Equation 2 in Appendix).

that may not be readily distinguished with the usual BSR methodology (Figures 7C and 8). Moreover, these findings can aid in explaining the inconsistency of prior reports. The traditional rate-frequency curves can be portrayed as 2D projections of a 3D structure. The face of the structure is diagonally oriented. Thus, when the mountain is displaced along an axis representing either pulse frequency or price, the 2D silhouette is displaced along the orthogonal axis. If the data are 2D, this can produce the illusion of motion in the plane in which the data are acquired when the actual movement was orthogonal to that plane. In other words, a shift along the price axis can create the illusion of a shift along the pulse-frequency axis. However, this relationship is asymmetrical. Note that the low slope of the diagonal portion of the contour lines in Figures 3, 5, & 7A implies that a given shift in P_e will produce a substantially smaller displacement in the silhouette of the mountain along the pulse-frequency axis, which is the sole independent-variable axis considered in traditional curve-shift experiments. Such shifts may not be discernable. Thus, it is not surprising that significant effects of CB1R blockade on ICSS have not been found in several prior studies (Vlachou et al., 2003, 2005; Xi et al., 2008). The detection problem is compounded by small shifts in F_{hm}, which can counteract the displacement of the 2D silhouette due to the shift of the 3D structure along the price axis. Moreover, the three reliable F_{hm} shifts observed here were inconsistent in sign. This reduces the likelihood of finding a significant effect when shifts in F_{m50} are averaged across subjects and group comparisons are carried out. In contrast, the 3D representation of single-subject results (Figure 5) renders the shifts and their statistical reliability unambiguously and clearly.

We were able to detect effects of CB1 receptor blockade on the pursuit of BSR by manipulating both the strength and cost of rewarding stimulation and by applying a 3D analysis appropriate for testing the influence of drugs on the performance of individual subjects; these effects would not have been seen using traditional curve-shift methods. The F_{hm} parameter reflects the sensitivity of the BSR substrate. It is defined as the stimulation strength required to produce a rewarding effect of half-maximal intensity. We found that although AM-251 does not produce consistent changes in this parameter, it does produce consistent decreases in willingness to pay for the stimulation, as reflected in the P_e parameter.

The mountain model highlights the danger of confining interpretation to a particular 2D plane. It is important to avoid this danger when interpreting the decreased willingness to pay. According to the model, a decrease in P_e can arise in multiple ways (Figure 1).

Although the leftward shift along the price axis could reflect increased subjective costs, it may also be explained otherwise, e.g., by an decrease in reward-system gain. Further methodological progress will be required to choose between the currently tenable explanations.

Depression has been linked to dopaminergic dysfunction and to a blunted reaction to rewards (Martin-Soelch, 2009). The latter symptom is consistent with a reduction in the gain of brain reward circuitry. Reduced gain in the BSR substrate is a tenable

explanation of the results reported here. In this regard, it is noteworthy that an increase in the incidence of depressed mood has been noted in participants in clinical trials of rimonabant (Moreira et al., 2009; Van Gaal et al., 2008), a CB1R antagonist.

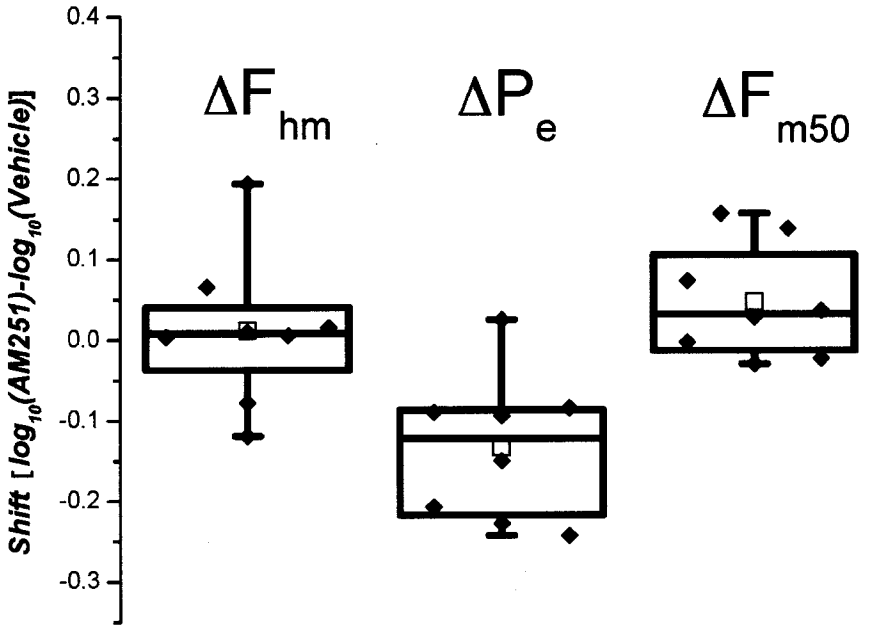

Figure 8. The mountain model can reveal shifts of cannabinoid receptor blockade that cannot be discerned with the conventional curve-shift method.
The effects of AM-251 administration on F_{hm}, P_e and the simulated F_{m50} values are shown. Each black diamond represents the estimated shift of the corresponding parameter for a given subject. Whiskers represent the maximum and minimum values, and the upper and lower borders of the box denote the 25th and 75th percentiles; the mean and the median are represented by the small inner square and the horizontal line, respectively. Note that the F_{hm} and F_{m50} shifts tend to group around zero whereas the P_e shifts are grouped around a mean shift of -0.13 common logarithmic units.

The allocation of behavior to the pursuit of reward necessarily depends on multiple variables, including reward strength, cost, probability, delay, and risk (Shizgal,

1997). Methods that can distinguish and quantify the contributions of these different variables will be required in order to determine the roles in reward seeking played by different neural systems. The findings reported here constitute one step toward understanding the contribution(s) of the endogenous cannabinoid system in the evaluation, selection, and pursuit of appetitive goals. The combination of quantitative modeling and multidimensional measurement of behavior promises future advances towards this goal.

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APPENDIX

Supplementary Equation1: The reward-mountain model

The quantitative model originally proposed by Arvanitogiannis and Shizgal (2008) and updated by Hernandez et al. (submitted) is represented in Equation 1. In this model, reward intensity is defined as the subjective strength of BSR and price as the time required to earn a train of rewarding stimulation (opportunity cost). The rewarding stimulation is assumed to consists of brief trains of current pulses.

$$TA(F,P) = TA_{\min} + \left((TA_{\max} - TA_{\min}) x \frac{\left(\frac{F^g}{F^g + F_{hm}^g} \right)^a}{\left(\frac{F^g}{F^g + F_{hm}^g} \right)^a + \left(\frac{P}{P_E} \right)^a} \right) \quad 1$$

where a determines the rate at which TA grows as the payoff from BSR increases,

F is the pulse frequency,

F_{hm} is the pulse frequency that produces half maximal reward,

g determines the rate at which reward intensity grows as F is increased,

P is the price of the stimulation train

and P_e is the price at which a maximum BSR equals the payoff from competing activities.

Supplementary Equation2: Obtaining F_{m50} values

The mountain model shown in Supplemenary Equation 1 was solved to calculate half-maximal performance at a given price. This yields equation 2:

$$Log_{10}(F_{m50}) = Log_{10}(F_{hm})\left[\frac{1}{g} x Log_{10}\left(\frac{P}{P_e - P}\right)\right] \qquad 2$$

where F_{m50} is the pulse frequency that produces half-maximal time allocation,

F_{hm} is the pulse frequency that produces half-maximal reward intensity,

g is the exponent (growth constant) of the intensity-growth function,

P is the price (opportunity cost) of the stimulation train,

and P_e is the price at which the rat devotes half of its time to harvesting a reward of maximal intensity.

As mentioned in the main text, F_{m50} was calculated for a price (P) of 0.1 s to account for the very low price of reward in curve-shifts experiments carried out with continuous reinforcement schedules. In that widely used method, reward is delivered upon a single lever press.