Published in final edited form as:

Neuropharmacology. 2012 September ; 63(4): 565–574. doi:10.1016/j.neuropharm.2012.04.036.

Cannabinoid Receptors Mediate Methamphetamine Induction of High Frequency Gamma Oscillations in the Nucleus Accumbens

Joshua T. Morra¹, Stanley D. Glick¹, and Joseph F. Cheer^{2,*}

¹47 New Scotland Avenue, Center for Neuropharmacology and Neuroscience, Albany Medical College, Albany, New York 12208

²Department of Anatomy and Neurobiology, Department of Psychiatry, University of Maryland School of Medicine, 20 Penn Street, Baltimore, Maryland 21201

Abstract

Patients suffering from amphetamine-induced psychosis display repetitive behaviors, partially alleviated by antipsychotics, which are reminiscent of rodent stereotypies. Due to recent evidence implicating endocannabinoid involvement in brain disorders, including psychosis, we studied the effects of endocannabinoid signaling on neuronal oscillations of rats exhibiting methamphetamine stereotypy. Neuronal network oscillations were recorded with multiple single electrode arrays aimed at the nucleus accumbens of freely moving rats. During the experiments, animals were dosed intravenously with the CB1 receptor antagonist rimonabant (0.3 mg/kg) or vehicle followed by an ascending dose regimen of methamphetamine (0.01, 0.1, 1, and 3 mg/kg; cumulative dosing). The effects of drug administration on stereotypy and local gamma oscillations were evaluated. Methamphetamine treatment significantly increased high frequency gamma oscillations (~ 80 Hz). Entrainment of a subpopulation of nucleus accumbens neurons to high frequency gamma was associated with stereotypy encoding in putative fast-spiking interneurons, but not in putative medium spiny neurons. The observed ability of methamphetamine to induce both stereotypy and high frequency gamma power was potently disrupted following CB1 receptor blockade. The present data suggest that CB1 receptor-dependent mechanisms are recruited by methamphetamine to modify striatal interneuron oscillations that accompany changes in psychomotor state, further supporting the link between endocannabinoids and schizophrenia spectrum disorders.

Introduction

Psychomotor activation is a classically described behavioral phenomenon observed in rodents administered amphetamines or other psychostimulants. Following low drug doses, psychomotor activation is characterized by increased locomotor behavior at the expense of grooming, feeding and other normal rodent behaviors [1]. Higher challenge doses elicit repetitive head movements, excessive sniffing and other stereotyped behaviors (stereotypies) [2]. Human patients suffering from amphetamine psychosis display repetitive, stereotyped behaviors similar to rodent stereotypy [3]. Moreover, both rodent [4] and human [5] stereotypies can be alleviated by treatment with typical antipsychotics. These observations

© 2012 Elsevier Ltd. All rights reserved.

*Correspondence: Joseph F. Cheer, Phone: (410) 706 0112, jchee001@umaryland.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

suggest that amphetamine-induced stereotypy may be an appropriate animal model for the psychomotor expression of psychosis [4].

Recent evidence suggests that cannabinoid CB1 receptor signaling mediates rodent stereotypy. Both wild-type rodents pretreated with CB1 receptor antagonists and CB1 KO mice express diminished amphetamine- [6] and methamphetamine- [7] induced stereotypy. Furthermore, CB1 receptor antagonist microinjections applied locally in the nucleus accumbens (NAc) reduce methamphetamine-induced stereotypy [7]. These findings implicate endocannabinoid(eCB) signaling in the ventral striatum as a crucial neural substrate for psychostimulant stereotypies.

Amphetamine doses capable of stereotypy induction have recently been shown to increase high frequency (~ 80 Hz), local field potential (LFP) gamma oscillations (γ) in the ventral striatum of behaving rodents [8]; however, the relationship between γ and stereotyped behavior is unknown. We recently demonstrated that neuronal firing patterns in the ventral striatum encode methamphetamine-induced stereotypy [7]. Moreover, this phenomenon was preferentially expressed in putatively identified fast spiking interneurons (FSIs). It is therefore possible that γ oscillations in the ventral striatum are a reflection of local, coordinated FSI activity required for stereotypy expression.

The current study aimed to examine the relationship between γ oscillations in the ventral striatum and the expression of methamphetamine-induced stereotypy. We show that methamphetamine dose-dependently increases high frequency (70-94 Hz, ~ 80 Hz peak) γ power (γ_{80}) in the NAc of freely-moving rats. Furthermore, we demonstrate that CB1 receptor signaling contributes to γ_{80} induction. Finally, we show that the extent to which FSIs cohere with local γ_{80} predicts that unit's propensity to encode stereotypy. Interestingly, this phenomenon was not recapitulated in putatively identified medium spiny neurons (MSNs). These findings further substantiate a role for ventral striatal FSIs in the mediation of eCB-dependent psychostimulant stereotypy.

Results

Methamphetamine Dose-Dependently Increases Gamma Power in the Nucleus Accumbens

Amphetamine has been shown to increase γ in the striatum [8]. To determine if other amphetamines (such as methamphetamine) possess γ -inducing properties, we recorded LFP activity in the ventral striatum following administration of saline, followed by methamphetamine (0.01, 0.1, 1 and 3 mg/kg; i.v.; cumulative dosing). Similar to amphetamine, methamphetamine dose-dependently increased γ LFP power in the NAc (Figure 1).

Rimonabant Attenuates Methamphetamine-Induced Increases in γ_{80}

We previously demonstrated that rimonabant attenuates methamphetamine-induced changes in firing rate in the ventral striatum [7]. To determine if CB1 receptor antagonism also curbs methamphetamine-induced γ_{80} , we recorded LFP activity in the NAc of behaving rats treated with either rimonabant (0.3 mg/g; i.v.; n = 4) or vehicle (n = 5), followed by saline and increasing doses of methamphetamine thereafter (0.01, 0.1, 1 and 3 mg/kg; i.v.; cumulative dosing). The single recording site with the greatest γ response in each animal was chosen for further analysis. Comparison of maximal response sites in the core and shell revealed that Nac sub-region had no effect on methamphetamine-induced changes in LFP frequencies between 0-100 Hz (data not shown; $F_{(1,127)} = 0.06$, $p > 0.05$). Therefore, maximal response sites from the core and shell were pooled for further analysis. Methamphetamine dose-dependently increased LFP power in the 70 to 94 Hz bandwidth (figure 2A; 2 way ANOVA, $F_{(4,127)} = 5.07$, $p < 0.001$; Bonferroni posttest p values ranging

from < 0.01 to < 0.001), but not LFP power across other bandwidths (figure 2A; Bonferroni posttest p values > 0.05) in vehicle-treated subjects. Therefore, subsequent analyses were limited to the γ_{80} bandwidth. Rimobant attenuated methamphetamine-induced γ_{80} (Figure 2B, 2 way ANOVA, $F_{(1,75)} = 3.02$, $p < 0.001$), whereas rimobant alone had no effect on γ_{80} power (Figure 2B, 1 way ANOVA, $F_{(1,75)} = 4.43$, $p > 0.05$).

Phasic Changes in γ_{80} do not Accompany Psychomotor Activation

We previously reported that phasic changes in NAc firing patterns encode the onset of stereotypy [7]. We show in Figure 3A that rimobant was effective in reducing methamphetamine-induced stereotypy (unpaired t test, $t_{(6)} = 3.36$, $* p < 0.05$), but not locomotion (unpaired t test, $t_{(6)} = 1.16$, $p > 0.05$). To determine if stereotypy initiation is temporally encoded by changes in γ_{80} we examined brief changes in γ_{80} time-locked to the onset of each behavioral bout. We observed phasic increases in γ_{80} both at the onset of stereotypy and throughout the remainder of each behavioral bout in a subset of experiments (Figure 3B). However, we failed to replicate this finding in our main pharmacological experiment, as neither rimobant nor vehicle-treated rats exhibited γ_{80} encoding of stereotypy (Figure 3C). To confirm that methamphetamine-induced γ_{80} represents a direct pharmacological effect on striatal circuitry and not an epiphenomenon secondary to changes in overall psychomotor state, we similarly examined changes in γ_{80} power time-locked to the onset of each bout of locomotion. As expected, we did not observe evidence of γ_{80} locomotor encoding (Figure 3D). However, this does not completely rule out that some of the changes in power may arise from a combination of motor and pharmacological effects.

Spiking Activity in the Nucleus Accumbens is Entrained to Gamma Oscillations

Our prior electrophysiological findings demonstrate that *local*, ventral striatal CB1 receptor signaling mediates stereotypy [7]. However, it is possible that LFP signals recorded in the NAc are generated by passive volume transmission of extrastriatal γ , rather than local neuronal activity. To determine if NAc γ is related to local neuronal activity, we examined the temporal relationship between γ and neuronal spiking activity simultaneously recorded in the ventral striatum.

We observed γ_{80} -entrained spiking activity in a subpopulation of putative medium spiny neurons (MSNs; Figures 4A, B and C; 14 out of 90 recorded neurons, 15.6%), fast-spiking interneurons (FSIs; Figures 4A, B and C; 1 out of 12 recorded neurons, 8.3%) and non-FSI interneurons (5 out of 20 recorded neurons, 25.0%, not shown). To confirm the validity of our averaged peri-spike waveforms, we compared these segments to surrogate segments generated when a random jitter (between -10 and 10 ms) was applied to the raw reference timestamps (Figures 4B and 4C). As expected, this jitter disrupted γ_{80} -spike coherence without altering entrainment to lower frequency waveforms. Subjectively identified entrainment was confirmed by objective statistical analysis using a custom bootstrap algorithm (data not shown; see Methods, Coherence).

Neither methamphetamine (for MSNs Figure 4E left inset; 1 way ANOVA, $F_{(1,1)} = 2.42$, $p > 0.05$; for FSIs Figure 4E right inset; 1 way ANOVA, $F_{(1,1)} = 1.66$, $p > 0.05$) nor rimobant (for MSNs Figure 4E left inset; 1 way ANOVA, $F_{(1,1)} = 0.45$, $p > 0.05$; for FSIs Figure 4E right inset; 1 way ANOVA, $F_{(1,1)} = 1.78$, $p > 0.05$) significantly altered neuronal coherence to the γ_{80} LFP bandwidth. To ensure that these negative findings were not due to the inclusion of neurons with relatively low γ_{80} coherence, a similar analysis was restricted to neurons exhibiting statistical criteria for γ_{80} coherence (see methods; data not shown; $n = 18$ after exclusion of the pilot experiment; no main effect for saline vs. methamphetamine, 1 way ANOVA, $F_{(1,1)} = 3.00$, $p > 0.05$; no main effect for vehicle vs. rimobant, 1 way ANOVA $F_{(1,1)} = 0.31$, $p > 0.5$).

However, when analysis was restricted to stereotypy-encoding units, a relationship was observed between γ_{80} -spike coherence and phasic neuronal encoding of stereotypy. The degree to which anFSI exhibited γ_{80} coherence was predictive of its propensity to effectively encode the onset of stereotypic behavior (Figure 5B right panel; linear regression, $F_{(1,6)} = 6.88$, $R^2 = 0.53$, $p < 0.05$). By contrast, this phenomenon was not recapitulated in MSNs (Figure 5B left panel; linear regression, $F_{(1,10)} = 0.23$, $R^2 = 0.02$, $p > 0.05$).

Striatal Gamma is Locally Generated

The existence of γ -entrained striatal neurons strongly suggests that striatal γ is generated locally. To confirm that these phenomena are local and not due to volume conduction within the striatum or from the nearby piriform cortex, we further examined γ oscillations at each recording site. We observed bandpassed (50-100 Hz), averaged LFP segments time-locked to γ waveforms peaks recorded at each electrode. This analysis failed to demonstrate any evidence of γ volume conduction, such as peak amplitude degradation as a function of inter-electrode distance or anatomical distance from the piriform cortex. Figure 6 shows a representative example of this analysis in an 8 electrode row implanted in the NAc of an animal given methamphetamine.

Discussion

Reconciling Differences in Psychostimulant Modulation of Gamma

γ oscillations have been proposed to serve as a mechanism by which appropriate neural ensemble spike timing is achieved in the mammalian brain [9]. γ is widely expressed across brain regions, and has been observed in numerous cortical [8,10,11] and subcortical [12] structures, including the striatum [13,14]. In humans, deficits in cortical γ coherence have been linked to several neuropsychiatric disorders, including schizophrenia [15,16]. It is possible that aberrant schizophrenic γ profiles are related to psychosis-associated hyperdopaminergia; however, few studies have explored this possibility.

Rodents studies reveal 2, functionally independent γ bandwidths, a low frequency γ bandwidth (30-70 Hz, peak at ~60 Hz) (γ_{60}) and the higher frequency γ_{80} [8, 17,18]. A recent study demonstrated that the psychotomimetic agents ketamine and MK-801 robustly enhance γ_{60} in the neocortex of behaving rats. In contrast, amphetamine or the direct dopamine (DA) agonist apomorphine only mildly increased γ_{60} [19]. However, a subsequent study examined γ over a wider bandwidth (40-100 Hz) in rats treated with either amphetamine or apomorphine [8]. Both DA agonists were shown to cause a dramatic power shift from γ_{60} to γ_{80} in the cortex and striatum of behaving rats [20].

The current study aimed to expand upon burgeoning work implicating the importance of γ in the NAc [18, 21]. We demonstrate that, unlike its congener amphetamine, methamphetamine profoundly increases γ_{80} without altering its low frequency counterpart. It has been suggested that γ_{60} is primarily associated with olfactory processing in the piriform cortex, whereas γ_{80} is likely related to frontal cortex activity [8]. Moreover, both brain regions send non-reciprocal efferents to the NAc [22,23] and exhibit high levels of striatal γ coherence [20]. Therefore, although the modulation of olfactory processing may differ between amphetamine and methamphetamine, both psychostimulants perturb coordinated frontostriatal activity during motivated behavior [13], which may underlie their similar psychotomimetic properties.

Cannabinoid Signaling Drives Neurophysiological Responses to Methamphetamine

We previously reported that eCB signaling drives methamphetamine-induced changes in NAc firing rates, and that this phenomenon is observed preferentially in putative FSIs [7].

Consistent with these findings, we now show that methamphetamine-induced NAc γ_{80} also requires CB1 transduction. This may indicate that γ_{80} is functionally related to local interneuron activity. Indeed, a subpopulation of FSIs has previously been reported to exhibit spiking activity phase locked to γ_{80} [8,13,24]. Likewise, we observed that a subpopulation of FSIs is entrained to γ_{80} . We additionally note that a subpopulation of MSNs and non-FSI interneurons also exhibit robust γ_{80} entrainment. Taken together, these findings suggest that eCB transmission in striatum may drive coordinated, γ_{80} -tuned neuronal activity. Likewise, our findings add to a growing literature suggesting that CB1 receptor activation is crucially important for network dynamics in the hippocampus and prefrontal cortex [25,26]. Our data confirm and extend these findings by showing that oscillatory brain activity is mediated by eCBs, whereas other studies in freely-moving animals have either failed to see an effect of CB1 receptor antagonists or have only used agonists to delineate an effect of CB1 receptor activation [25,26].

Congruent with aforementioned theoretical frameworks attributing γ_{80} to cortical efferent activity, methamphetamine increases corticostriatal glutamate release in the NAc [27]. Augmented efferent drive could induce coordinated high frequency oscillations in target striatal neurons, which is ultimately reflected by extracellular γ_{80} . eCBs could play a permissive role in this sequence. Briefly, CB1 receptor stimulation curbs both glutamatergic and GABAergic neurotransmission [28,29,30,31]; however, in the striatum, GABAergic synapses are more sensitive to cannabinoid inhibition than their excitatory counterparts [32]. It is possible that endogenous CB1 tone acts to dampen inhibitory drive onto corticostriatal neuronal targets, thereby allowing high-frequency excitatory input to dominate their oscillatory patterns. Alternatively, precisely timed CB1 receptor signaling on corticostriatal terminals themselves may tune presynaptic glutamate release such that high frequency, post-synaptic reverberations occur. Further investigation is clearly required if these speculations are to be delineated.

Surprisingly, neither methamphetamine nor rimonabant altered gross neuronal γ_{80} entrainment. By definition, γ_{80} spike coherence is more readily identified as the analysis incorporates increasing numbers of spikes. This consideration is particularly relevant to striatal units, which typically have low firing rates and γ coherence. It is therefore foreseeable that rimonabant alters γ_{80} power without altering spike coherence to this frequency band. Since rimonabant is presumably working at presynaptic receptors, its effect is most easily accounted for by presuming that rimonabant interferes with γ_{80} -entrained, presynaptic neurotransmitter release, rather than by directly affecting post-synaptic fluctuations in membrane potential that are already γ -entrained. Because rimonabant has the potential to alter presynaptic signal transduction, without directly affecting post-synaptic properties, post-synaptic entrainment to a residual γ_{80} signal should not be modulated by rimonabant. Therefore, the degree of γ_{80} entrainment may be a good predictor for how much stereotypy-relevant information a given neuron is receiving as compared to other neurons, but a poor predictor for how much stereotypy is being expressed behaviorally (which is indeed what we observed).

That methamphetamine fails to increase γ_{80} spike coherence, despite its robust effect on overall γ_{80} power, is puzzling. Perhaps methamphetamine augments striatal γ not through local dopaminergic effects, but by promoting γ_{80} oscillations in non-striatal brain regions from which the γ_{80} signal originates (i.e. the prefrontal cortex). Such a mechanism would allow methamphetamine to have robust effects on gross γ_{80} generation without altering striatal coherence to synaptic input across the γ_{80} bandwidth. Future investigations of this matter are justified.

Striatal Gamma-Spike Coherence is Associated with Stereotypy Encoding

Phasic changes in ventral striatal firing patterns have been shown to encode the onset of stereotypy in methamphetamine-treated animals [7]. In contrast, we show that fluctuations in γ_{80} on an identical timescale fail to encode stereotypic initiation. These negative findings are not entirely unexpected given previous reports demonstrating that phasic increases in γ_{60} , but not γ_{80} , are associated with movement initiation [17, 33]. Nevertheless, the finding that γ_{80} entrainment predicts neuronal stereotypy encoding strength suggests that information required to select behaviorally relevant interneuron ensembles is transmitted across the γ_{80} bandwidth. That the correlation coefficient for this relationship is low is to be expected; γ_{80} oscillations are in large part an indication of the frequency at which the cortex relays information to the striatum. Therefore vast disparate data are no doubt embedded within this bandwidth, with stereotypy-related information comprising only a fraction of the total corticostriatal information mass. A similar line of thought may explain why fluctuations in overall γ_{80} power fail to encode the onset of stereotypy. It is possible that total corticostriatal information flow, and its putative index γ_{80} , remain unchanged during stereotypy, while the portion of γ_{80} signal pertinent to stereotypic behavior increases relative to its irrelevant counterparts during such behavioral bouts.

Building a Case for the Preferential Involvement of Fast-Spiking Interneurons in Stereotypy

We previously published that FSIs, as opposed to MSNs, preferentially encode the onset of methamphetamine stereotypy [7]. Moreover, multiple research groups have reported γ_{80} entrainment of striatal FSI spiking activity [8, 9, 12-14]. When our findings are considered within this context, it seems reasonable to posit that γ_{80} -entrained FSI activity contributes to stereotypy. Indeed, we observed γ_{80} entrainment in both MSNs and FSIs; however, only FSI entrainment was shown to correlate with a neuron's propensity to relay stereotypy-pertinent information. It should be noted that this observation likely relied upon small subpopulations of heavily entrained neurons, suggesting that any interpretation rendered here is best entertained with caution. That said, our findings may suggest that FSIs, rather than MSNs, are preferentially tuned to the stereotypy.

This can perhaps be explained by observed differences in afferent innervation to these two striatal populations. Specifically, it is known that glutamatergic drive excites discrete MSN ensembles through monosynaptic transmission and can silence other MSNs through distal activation of FSIs [34, 35, 36, 37]. Our results support the notion that an organization of feedforward inhibition exists, in which corticostriatal input to one site of the FSI network may relay excitation to other FSIs within the ensemble to produce a selective inhibitory influence on MSNs situated at other loci within the same microcircuit. A release of FSI-mediated inhibition can then function as a gating mechanism for MSN action and perhaps this occurs through FSI γ_{80} -entrained input, leading ultimately to the production of stereotypy.

Psychosis May Recruit Endogenous Cannabinoid Signaling

Human studies implicate a role for eCB signaling in psychosis. Recent reports have linked a 9-time AAT triplet repeat in the 3' region of the CNR1 gene, the gene encoding the human CB1 receptor, to the development of hebephrenic schizophrenia [40, 41, 42]. Moreover, serum [41, 43] and cerebrospinal fluid [41, 44, 45] levels of the eCB anandamide are elevated in unmedicated, first episode paranoid schizophrenic patients, while levels are normalized in schizophrenic patients receiving typical antipsychotics [41, 45].

Animal studies confirm the importance of eCB signaling in psychosis-related behavioral paradigms. Deficits in prepulse inhibition induced by ketamine or MK-801 are ameliorated

by CB1 receptor antagonists [46,47,48]. Likewise, CB1 KO mice [6] and wild-type rodents treated with CB1 antagonists [6,7] exhibit reduced psychostimulant stereotypy (although see Martin et al. 2003 [4]). Moreover, local CB1 receptor blockade in the NAc also attenuates stereotypy, implicating the ventral striatum as a fundamental neuroanatomical substrate of cannabinoid-mediated psychosis [7]. This is consistent with our current work, which shows that stereotypy encoding accompanies cannabinoid receptor-mediated γ . Future experiments should examine whether antipsychotics prevent methamphetamine-induced LFP gamma oscillations and whether CB1 receptor agonists (direct or indirect) increase accumbal γ power. Together, the novel findings presented here support a growing body of literature suggesting that CB1 receptor antagonists may possess clinically relevant antipsychotic properties.

Materials and Methods

All experiments were performed according to United States Public Health Service Guide for the Care and Use of Laboratory Animals and were approved by the Albany Medical College Institutional Animal Care and Use Committee and the University of Maryland School of Medicine Institutional Animal Care and Use Committee.

Animals and Surgery

Adult, male Sprague Dawley rats ($n = 10$; 300-400 g; Charles River) were individually housed in a 12 hr light/dark environment. Animals were given *ad libitum* access to food and water. Each animal was surgically implanted with a unilateral, 8×2 stainless steel microelectrode array (700 k Ω impedance as measured in saline with a 100 Hz sine wave input; Microprobe Inc.) as previously described [7]. Ground electrodes were implanted in the contralateral cortex. Subjects were permitted to recover from surgery for 1 week prior to experiments.

Electrophysiological Recordings

LFP and multiple single-unit activity were simultaneously recorded in awake, behaving rats. Animals were connected to a flexible cable with a printed circuit board headstage containing a micro-operation amplifier for individual unity gain amplification from each electrode. Signals were routed to a differential preamplifier (fixed $50 \times$ gain; Plexon Inc.) and a relayed Multineuron Acquisition Processor (Plexon Inc.) which allows for computer-controlled, channel-specific signal amplification (gain steps 1-30, total gain $1000 \times$ to 32000 $\times$), filtering (second-order 500 Hz low cut, 5 kHz high cut) and analog to digital conversion (32 simultaneous sampling 12-bit converters, 75 kHz). Multiunit signals were referenced to ground, sorted from noise online and spike sorted offline as previously described [7]. LFP signals were referenced to ground on a skull screw in the contralateral occipital bone and collected at a 1 kHz sampling frequency. All raw LFP voltage traces and whole-session power spectral densities (PSDs) were visually inspected to confirm recording quality prior to further analysis.

A subset of recording sites exhibited brief, sporadic bouts of high amplitude 60 Hz noise artifact. To exclude such channels from further analyses could introduce selection bias into our data by which channels exhibiting prominent low frequency γ would be discounted. We instead employed a signal processing strategy through an offline, custom-written (Matlab) artifact filter, which reduced oscillatory signals > 6 to 8 standard deviations from the mean signal to the mean LFP value. This outlier cut-off was guided by signal gain settings used for a given channel, and determined to address high amplitude anomalies, without disrupting lower amplitude 60 Hz signals. Examination of pre- and post-filtered PSDs revealed that this manipulation removed sharp peaks in the PSD at 60 Hz without altering non-60 Hz

bandwidths. Comparison of filtered PSDs to naturally artifact-free PSDs revealed comparable peaks at 60 Hz, confirming that biologically relevant 60 Hz oscillations remained unfiltered.

Dosing Regimen

Rats were individually habituated daily for 3 days to a custom-modified, sound-attenuated activity chamber (Med Associates) equipped with a rotating commutator to allow for electrophysiological recording and intravenous drug administration during ongoing behavior. On the fourth day, animals were subjected to 20 min of baseline recording. Rats were then administered either rimonabant (0.3 mg/kg; i.v.; $n = 4$), vehicle ($n = 5$) or no treatment ($n = 1$) followed 5 min later by saline (i.v.) and increasing doses of methamphetamine thereafter (0.01, 0.1, 1, 3 mg/kg; i.v.; cumulative dosing; 5 min inter-dose interval). Subjects were then permitted to behave freely until their motor behavior returned to pre-drug values (~ 3 -6 hrs).

Behavior

Stereotypy was quantified by a experimenter blinded to treatment as previously described [7]. Briefly, stereotypy was scored as either “present” or “not present” in 1 s bins for the first 75 min of each experiment. A score of “present” was assigned when each of 3 criteria was satisfied: (1) all 4 of the rats paws were stationary, (2) the animal was excessively sniffing and (3) the subject performed at least 3 rhythmic (~ 4 -8 Hz) head bobs. Each stereotypy bout began at the onset of the first rhythmic head bob.

Neuron Classification

Striatal neurons were sorted based on their spike waveform shape and firing rates as previously described [7]. In short, neurons with relatively slow spike waveforms (peak width at half maximum $> 120 \mu\text{s}$; valley width at half minimum $> 165 \mu\text{s}$) and low firing rates ($< 5 \text{ Hz}$) were classified as putative medium spiny neurons (MSNs). Neurons with relatively fast spike waveforms (peak width at half maximum $< 120 \mu\text{s}$; valley width at half minimum $< 165 \mu\text{s}$) were classified as putative fast-spiking interneurons (FSIs). Statistical analyses excluded neurons which failed to meet the criteria for either MSNs or FSIs.

Neuronal Encoding of Behavior

Neuronal encoding strength was determined as previously described [7]. Briefly, peri-event raster plots about the onset of either locomotion or stereotypy ($\pm 2 \text{ s}$, 100 ms bins) were constructed for each recorded neuron. Peri-event histograms, with firing rates normalized to baseline (-2 to -1 s), were averaged across 15 to 60 trials, depending on the number of high quality behavioral trials that could be isolated in a given animal (see Figure 5A). Only trials in which the criteria for locomotion and stereotypy were *not* met for a full 2 seconds prior to behavioral initiation were included. Locomotion trials were restricted to behavior occurring after the administration of 0.1 mg/kg methamphetamine, whereas stereotypy trials were restricted to onset times following 3 mg/kg. Neurons whose mean peri-event firing rates exceeded an absolute z score 2 between -1 and $+1 \text{ s}$ were said to encode the behavior of interest. Mean z scores at time 0 were designated as a neuron’s encoding “strength”.

Power Spectral Densities

LFP rate histograms and standard 2-dimension PSDs were calculated and graphed using NeuroExplorer (Nex Technologies). Power expressed in dB was derived as the $\log_{10}$ of the raw PSD. Power spectrograms were calculated and graphed using custom-written MATLAB scripts (The Mathworks). Whole-session power spectrograms were restricted to frequencies from 0 to 100 Hz and were calculated using a 1 s Hamming window with a 1 s step size.

Peri-event power spectrograms were restricted to frequencies from 0 to 100 Hz and were calculated using a 100 ms Hamming window with a 50 ms step size. Changes in high γ power over time were calculated by averaging the PSD from 50 to 100 Hz using a 50ms Hamming window with a 50 ms step size.

Coherence

LFP-spike coherence was calculated and graphed using NeuroExplorer, which employs the following equation for coherence:

$$C_{xy} = (P_{xy} * P_{xy}) / (P_{xx} * P_{yy}) \quad (1)$$

where C_{xy} equals coherence between variables x and y, P_{xx} equals the average of the squared spectra of x, P_{yy} equals the average of the squared spectra of y and P_{xy} equals the product of the spectra for x and y.

For each recorded unit, γ entrainment was statistically determined using a custom bootstrap Matlab function, similar to functions employed in previously published reports [17]. Briefly, for each recorded spike train, inter-spike intervals were randomly shuffled 100 times to create 100 surrogate spike trains with identical inter-spike interval histograms and mean firing rates, but randomly varying spike distributions with respect to local γ . Spike-LFP coherence at 80 Hz for each actual spike train was then converted to a z score referenced to its surrogates' coherence distribution. A z score greater than 3 was said to indicate significant high γ coherence. Spike-field coherence peak values at 80 Hz were used for any further analyses of high γ spike coherence.

Histology

Electrode tip placement was confirmed as previously described [7]. Signals recorded from electrodes positioned outside the NAc were excluded from further analysis.

Data Analysis and Statistics

Bar graphs, line graphs, scatter plots were created and all statistical analyses were performed using Prism 5 (GraphPad Software). Statistical tests included standard 1 and 2 way ANOVAs followed by Tukey's multiple-comparisons test or Bonferroni posttests. The criterion for significance was set to $p < 0.05$. All error bars represent $\pm$ SEM. Linear regression and peri-event analyses used to assess the relationship between γ and stereotypy were restricted to behavioral timestamps and time bins following 3 mg/kg methamphetamine, as this dose was previously shown to produce robust stereotypy [7].

Drugs

Methamphetamine (Sigma-Aldrich) was delivered in saline. Rimonabant (SR141716A; Research Triangle Institute – National Institute on Drug Abuse) was delivered in a 1:1:18 ratio of ethanol, Emulphor and saline.

Acknowledgments

We would like to thank Joseph E. Mazurkiewicz and Marilyn Dockum for their assistance and technical expertise regarding histological methods. We also thank Joshua D. Berke for his helpful discussion regarding electrophysiological data analysis.

References

1. Pawlak AP, Tang CC, Pederson C, Wolske MB, West MO. Acute effects of cocaine on movement-related firing of dorsolateral striatal neurons depend on predrug firing rate and dose. *J Pharmacol Exp Ther.* 2010; 332:667–683. [PubMed: 19906778]
2. Kokkinidis L, Anisman H. Amphetamine psychosis and schizophrenia: a dual model. *Neurosci Biobehav Rev.* 1981; 5:449–461. [PubMed: 7033842]
3. Schiörring E. Psychopathology induced by “speed drugs”. *Pharmacol Biochem Behav.* 1981; 14(Suppl 1):109–122. [PubMed: 7243851]
4. Martin RS, Secchi RL, Sung E, Lemaire M, Bonhaus DW, et al. Effects of cannabinoid receptor ligands on psychosis-relevant behavior models in the rat. *Psychopharmacology (Berl).* 2003; 165:128–135. [PubMed: 12404071]
5. Grelotti DJ, Kanayama G, Pope HG Jr. Remission of persistent methamphetamine-induced psychosis after electroconvulsive therapy: presentation of a case and review of the literature. *Am J Psychiatry.* 167:17–23. [PubMed: 20068123]
6. Tzavara ET, Degroot A, Wade MR, Davis RJ, Nomikos GG. CB1 receptor knockout mice are hyporesponsive to the behavior-stimulating actions of d-amphetamine: role of mGlu5 receptors. *Eur Neuropsychopharmacol.* 2009; 19:196–204. [PubMed: 19116182]
7. Morra JT, Glick SD, Cheer JF. Neural encoding of psychomotor activation in the nucleus accumbens core, but not the shell, requires cannabinoid receptor signaling. *J Neurosci.* 2010; 30:5102–5107. [PubMed: 20371830]
8. Berke JD. Fast oscillations in cortical-striatal networks switch frequency following rewarding events and stimulant drugs. *Eur J Neurosci.* 2009
9. Lisman J, Buzsáki G. A neural coding scheme formed by the combined function of gamma and theta oscillations. *Schizophr Bull.* 2008; 34:974–980. [PubMed: 18559405]
10. Nase G, Singer W, Monyer H, Engel AK. Features of neuronal synchrony in mouse visual cortex. *J Neurophysiol.* 2003; 90:1115–1123. [PubMed: 12702711]
11. Hakami T, Jones NC, Tolmacheva EA, Gaudias J, Chaumont J, et al. NMDA receptor hypofunction leads to generalized and persistent aberrant gamma oscillations independent of hyperlocomotion and the state of consciousness. *PLoS One.* 2009; 4:e6755. [PubMed: 19707548]
12. Brown P, Kupsch A, Magill PJ, Sharott A, Harnack D, et al. Oscillatory local field potentials recorded from the subthalamic nucleus of the alert rat. *Exp Neurol.* 2002; 177:581–585. [PubMed: 12429204]
13. Berke JD, Okatan M, Skurski J, Eichenbaum HB. Oscillatory entrainment of striatal neurons in freely moving rats. *Neuron.* 2004; 43:883–896. [PubMed: 15363398]
14. Masimore B, Kakalios J, Redish AD. Measuring fundamental frequencies in local field potentials. *J Neurosci Methods.* 2004; 138:97–105. [PubMed: 15325117]
15. Jones MW. Errant ensembles: dysfunctional neuronal network dynamics in schizophrenia. *Biochem Soc Trans.* 2010; 38:516–521. [PubMed: 20298213]
16. Spencer KM, Nestor PG, Niznikiewicz MA, Salisbury DF, Shenton ME, et al. Abnormal neural synchrony in schizophrenia. *J Neurosci.* 2003; 23:7407–7411. [PubMed: 12917376]
17. van der Meer MA, Redish AD. Low and high gamma oscillations in rat ventral striatum have distinct relationships to behavior, reward and spiking activity on a learned spatial decision task. *Front Integr Neurosci.* 2009; 3:9. [PubMed: 19562092]
18. Howe MW, Atallah HE, McCool A, Gibson DJ, Graybiel AM. Habit learning is associated with major shifts in frequencies of oscillatory activity and synchronized spike firing in the striatum. *Proc Natl Acad Sci U S A.* 2011; 108:16801–6. [PubMed: 21949388]
19. Pinault D. N-methyl d-aspartate receptor antagonists ketamine and MK-801 induce wake-related aberrant gamma oscillations in the rat neocortex. *Biol Psychiatry.* 2008; 63:730–735. [PubMed: 18022604]
20. Berke JD, Breck JT, Eichenbaum H. Striatal versus hippocampal representations during win-stay maze performance. *J Neurophysiol.* 2009; 101:1575–1587. [PubMed: 19144741]

21. van der Meer MA, Kalenscher T, Lansink CS, Pennartz CM, Berke JD, Redish AD. Integrating early results on ventral striatal gamma oscillations in the rat. *Front Neurosci.* 2010; 4:300. [PubMed: 21350600]
22. Meyer DK, Beinfeld MC, Oertel WH, Brownstein MJ. Origin of the cholecystokinin-containing fibers in the rat caudatoputamen. *Science.* 1982; 215:187–188. [PubMed: 7053570]
23. Lapper SR, Bolam JP. Input from the frontal cortex and the parafascicular nucleus to cholinergic interneurons in the dorsal striatum of the rat. *Neuroscience.* 1992; 51:533–545. [PubMed: 1488113]
24. Sharott A, Moll CK, Engler G, Denker M, Grun S, et al. Different subtypes of striatal neurons are selectively modulated by cortical oscillations. *J Neurosci.* 2009; 29:4571–4585. [PubMed: 19357282]
25. Robbe D, Montgomery SM, Thome A, Rueda-Orozco PE, McNaughton BL, Buzsaki G. Cannabinoids reveal importance of spike timing coordination in hippocampal function. *Nat Neurosci.* 2006; 12:1526–33. [PubMed: 17115043]
26. Kucewicz MT, Tricklebank MD, Bogacz R, Jones MW. Dysfunctional prefrontal cortical network activity and interactions following cannabinoid receptor activation. *J Neurosci.* 2011; 31:15560–8. [PubMed: 22031901]
27. Zhang Y, Loonam TM, Noailles PA, Angulo JA. Comparison of cocaine- and methamphetamine-evoked dopamine and glutamate overflow in somatodendritic and terminal field regions of the rat brain during acute, chronic, and early withdrawal conditions. *Ann N Y Acad Sci.* 2001; 937:93–120. [PubMed: 11458542]
28. Gerdeman GL, Ronesi J, Lovinger DM. Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. *Nat Neurosci.* 2002; 5:446–451. [PubMed: 11976704]
29. Piomelli D. The molecular logic of endocannabinoid signalling. *Nat Rev Neurosci.* 2003; 4:873–884. [PubMed: 14595399]
30. Robbe D, Kopf M, Remaury A, Bockaert J, Manzoni OJ. Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. *Proc Natl Acad Sci U S A.* 2002; 99:8384–8388. [PubMed: 12060781]
31. Hoffman AF, Lupica CR. Direct actions of cannabinoids on synaptic transmission in the nucleus accumbens: a comparison with opioids. *J Neurophysiol.* 2001; 85:72–83. [PubMed: 11152707]
32. Adermark L, Lovinger DM. Frequency-dependent inversion of net striatal output by endocannabinoid-dependent plasticity at different synaptic inputs. *J Neurosci.* 2009; 29:1375–1380. [PubMed: 19193884]
33. Masimore B, Schmitzer-Torbert NC, Kakalios J, Redish AD. Transient striatal gamma local field potentials signal movement initiation in rats. *Neuroreport.* 2005; 16:2021–4. [PubMed: 16317346]
34. Kita H, Kosaka T, Heizmann CW. Parvalbumin-immunoreactive neurons in the rat neostriatum: a light and electron microscopy study. *Brain Res.* 1990; 536:1–15. [PubMed: 2085740]
35. Pennartz CM, Kitai ST. Hippocampal inputs to identified neurons in an in vitro slice preparation of the rat nucleus accumbens: evidence for feed-forward inhibition. *J Neurosci.* 1991; 11:2838–47. [PubMed: 1679123]
36. Bennett BD, Bolam JP. Synaptic input and output of parvalbumin-immunoreactive neurons in the neostriatum of the rat. *Neuroscience.* 1994; 62:707–19. [PubMed: 7870301]
37. Plenz D, Kitai ST. Up and down states in striatal medium spiny neurons simultaneously recorded with spontaneous activity in fast-spiking interneurons studied in cortex-striatum-substantia nigra organotypic cultures. *J Neurosci.* 1998; 18:266–83. [PubMed: 9412506]
38. Robbins TW, Iversen SD. Amphetamine-induced disruption of temporal discrimination by response disinhibition. *Nat New Biol.* 1973; 245:191–192. [PubMed: 4517785]
39. Robbins T, Iversen SD. A dissociation of the effects of d-amphetamine on locomotor activity and exploration in rats. *Psychopharmacologia.* 1973; 28:155–164. [PubMed: 4694624]
40. Ujike H, Takaki M, Nakata K, Tanaka Y, Takeda T, et al. CNR1, central cannabinoid receptor gene, associated with susceptibility to hebephrenic schizophrenia. *Mol Psychiatry.* 2002; 7:515–518. [PubMed: 12082570]

41. Fernandez-Espejo E, Viveros MP, Nunez L, Ellenbroek BA, Rodriguez de Fonseca F. Role of cannabis and endocannabinoids in the genesis of schizophrenia. *Psychopharmacology (Berl)*. 2009; 206:531–549. [PubMed: 19629449]
42. Chavarria-Siles I, Contreras-Rojas J, Hare E, Walss-Bass C, Quezada P, et al. Cannabinoid receptor 1 gene (CNR1) and susceptibility to a quantitative phenotype for hebephrenic schizophrenia. *Am J Med Genet B Neuropsychiatr Genet*. 2008; 147:279–284. [PubMed: 18186055]
43. De Marchi N, De Petrocellis L, Orlando P, Daniele F, Fezza F, et al. Endocannabinoid signalling in the blood of patients with schizophrenia. *Lipids Health Dis*. 2003; 2:5. [PubMed: 12969514]
44. Leweke FM, Giuffrida A, Koethe D, Schreiber D, Nolden BM, et al. Anandamide levels in cerebrospinal fluid of first-episode schizophrenic patients: Impact of cannabis use. *Schizophr Res*. 2007
45. Giuffrida A, Leweke FM, Gerth CW, Schreiber D, Koethe D, et al. Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. *Neuropsychopharmacology*. 2004; 29:2108–2114. [PubMed: 15354183]
46. Schneider M, Koch M. The cannabinoid agonist WIN 55,212-2 reduces sensorimotor gating and recognition memory in rats. *Behav Pharmacol*. 2002; 13:29–37. [PubMed: 11990717]
47. Fernandez-Espejo E, Galan-Rodriguez B. Sensorimotor gating in mice is disrupted after AM404, an anandamide reuptake and degradation inhibitor. *Psychopharmacology (Berl)*. 2004; 175:220–224. [PubMed: 15088080]
48. Makris N, Oscar-Berman M, Jaffin SK, Hodge SM, Kennedy DN, et al. Decreased Volume of the Brain Reward System in Alcoholism. *Biol Psychiatry*. 2008

Highlights

- Methamphetamine increases high frequency gamma oscillations in the accumbens
- These effects are attenuated by CB1 receptor blockade
- The endocannabinoid system is required for accumbal ensemble response to methamphetamine

Figure 1.

Methamphetamine increases LFP γ oscillations in the NAc. **(A, top)** Power spectrogram from a representative recording site in a subject given saline followed by cumulative methamphetamine administration (0.01, 0.1, 1 and 3 mg/kg; i.v.). All PSD values normalized from 0 to 1 (color scale). **(A, bottom)** Raw LFP data from the same experiment recorded following saline (left) and methamphetamine (right). Note the prominent increase in fast oscillations following methamphetamine. **(B)** PSDs for the raw traces displayed in (A), demonstrating the methamphetamine-induced enhancement of γ power.

Figure 2.

Methamphetamine-induced increases in γ_{80} require CB1 receptor activation. **(A)** Mean PSDs, normalized to baseline values, for LFPs at a single recording site in subjects given vehicle followed by saline (sal), followed thereafter by methamphetamine (0.01, 0.1, 1 and 3 mg/kg; meth). Note the dose-dependent increase in power across the high frequency γ (70 – 94 Hz) bandwidth (**, ***, 2 way ANOVA, $F_{(4,127)} = 5.07$, $p < 0.001$, Bonferroni posttest p values ranging from < 0.01 to < 0.001). Methamphetamine fails to alter LFP power across other bandwidths (Bonferroni posttest p values > 0.05). Error bars excluded for clarity. **(B)** γ_{80} across time in both veh- and rimo-treated subjects (1 min bins). Meth significantly augments γ_{80} (1 way ANOVA, $F_{(12,75)} = 7.13$, $p < 0.001$). Rimo pretreatment significantly attenuates meth-induced γ_{80} (2 way ANOVA, $F_{(1,75)} = 3.02$, $p < 0.001$). Error bars $\pm$ SEM.

Figure 3.

Phasic changes in γ_{80} are not associated with onset of stereotypy. **(A)** Stereotypy (top) and locomotion (bottom) expressed following methamphetamine (3 and 1 mg/kg; i.v., respectively) in vehicle- and rimonabant-treated subjects. Systemic rimonabant (0.3 mg/kg, i.v.) significantly attenuates stereotypy compared to vehicle-treated animals (unpaired t test, $t_{(6)} = 3.36$, $* p < 0.05$). Rimonabant fails to curb methamphetamine-induced locomotion (unpaired t test, $t_{(6)} = 1.16$, $p > 0.05$). **(B)** Mean power spectrogram for peri-event LFPs centered at the onset of individual bouts of stereotypy from a single recording site in an animal given saline followed by methamphetamine (0.01, 0.1, 1 and 3 mg/kg) (**top**). Mean LFP power is represented by the color scale; peri-event changes in γ power are depicted by the white line plot overlay. Brief fluctuations in γ appear to encode the onset of stereotypy (**bottom**). **(C)** Mean peri-event γ_{80} patterns averaged across all recording sites in vehicle- and rimonabant-treated subjects reveal that phasic changes in γ_{80} are maintained at the onset of neither stereotypy nor locomotion in either treatment group. Error bars $\pm$ SEM.

Figure 4.

A subpopulation of NAc neurons exhibit spiking activity entrained to γ_{80} oscillations. **(A)** Inter-spike interval histograms for a representative MSN (left) and FSI (right). Spike waveform shapes for the entire recording session shown in the insets. **(B)** Corresponding autocorrelograms for the units shown in (A). **(C)** Mean LFP segments time-locked to neuronal spiking activity for the units displayed in (A). If spiking activity and LFP activity occur irrespective of one another, averaging peri-spike LFP segments across large numbers of trials theoretically yields a flat line. Note the prominent peri-spike LFP waveforms. To confirm the validity of this analysis, we compared averaged peri-spike waveforms derived from raw data to similar waveforms derived using surrogate spike timestamps artificially jittered between -10 and $+10$ ms. **(D)** PSDs for mean peri-spike LFP segments displayed in (B). The robust peak at ~ 70 to 80 Hz suggests that the above peri-spike waveforms contain a significant γ component. Note that spike jitter interferes with γ entrainment, but not with lower frequency coherence. **(E)** Spike-LFP coherence for neurons displayed in (A). Note the peak in coherence in the γ bandwidth, confirming that these neurons exhibit γ -entrained spiking activity. Histogram insets depict changes in spike- γ_{80} coherence following both saline (sal) and methamphetamine (meth) in both vehicle- (veh) and rimonabant- (rimo) treated animals. Pharmacological manipulations had no gross effect on γ_{80} -spike coherence in MSNs (left inset; methamphetamine vs. saline, 1 way ANOVA, $F(1,1) = 2.42$, $p > 0.05$;

vehicle vs. rimonabant, 1 way ANOVA, $F(1,1) = 0.45$, $p > 0.05$) or FSIs (right insert; methamphetamine vs. saline, 1 way ANOVA, $F(1,1) = 1.66$, $p > 0.05$; vehicle vs. rimonabant, 1 way ANOVA, $F(1,1) = 1.78$, $p > 0.05$).

Figure 5.

FSI, but not MSN, gamma entrainment is associated with neuronal encoding of stereotypy. **(A)** Peri-event histograms of 2 representative neurons (MSN and FSI, left and right, respectively) encoding the onset of stereotypy. Line plot overlays depicting normalized peri-event firing rates allow for comparison of behavioral encoding strength (z score peak heights) between neurons. **(B)** Linear regression of stereotypy encoding strength as a function of γ_{80} coherence in stereotypy-encoding MSNs (left) and FSIs (right). γ_{80} and encoding strength are directly correlated across all treatment groups in FSIs (linear regression, $F_{(1,6)} = 6.99$, $R^2 = 0.54$, $p < 0.05$), but not MSNs (linear regression, $F_{(1,10)} = 0.23$, $R^2 = 0.22$, $p > 0.05$).

Figure 6.

Ventral striatal γ_{80} is locally generated. **(A)** A representative segment of LFP data before (raw) and after (γ_{80} bandpass) undergoing frequency filtering to isolate γ_{80} oscillations for further manipulation. Associated PSDs are shown. **(B)** LFPs recorded from a row of 8 electrodes in the NAc core during a representative experiment are shown (right). Data was bandpassed (70-94 Hz) and averaged about waveform peaks identified at the electrode most proximal to the nearby piriform cortex. The γ_{80} phase and amplitude relationship at each recording site as a function of anatomical proximity (left). Note that γ_{80} phase does not shift, nor does amplitude degrade as a function of inter-electrode distance, suggesting that high γ_{80} coherence between recording sites is attributed to common neurotransmissive influence, rather than passive volume conduction. Moreover, no evidence of volume conduction as a function of anatomic distance to the piriform cortex is observed.