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Interindividual variation in frontostriatal circuit dynamics correlates to degree of neural criticality and predicts cocaine cue-evoked behavioral arousal during early abstinence

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A dissertation submitted in partial satisfaction of the

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in Neuroscience

by

Wesley Charles Smith

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ABSTRACT OF THE DISSERTATION

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by

Wesley Charles Smith

Doctor of Philosophy in Neuroscience

University of California, Los Angeles, 2018

Professor Sotirios Masmanidis, Chair

Drugs of abuse and the environmental contextual stimuli that can predict their availability have long been known to influence behavior, leading to risk of relapse, one of the most difficult challenges facing those addicted to drugs. Addiction is thought to supplant the basal ganglia of the brain and its input structures, such that the concerted effort of the reward system is to make choices to obtain drugs, with environmental cues and internal states serving as driving forces. Because the phenomenon of addiction is rooted in interacting elements of reward circuits that involve many millions of neurons, as of yet, the neurophysiological correlates of the response of such large populations of neurons across the basal ganglia *in vivo* to conditioned drug cues and their functional interactions at rest are poorly understood. Although recording many neurons at once has become easier in recent years, the crux of the problem is finding meaning in such large datasets, especially in association with behavior. This dissertation is the first to examine frontostriatal circuits in addiction by recording *in vivo* from hundreds of neurons simultaneously in mouse medial prefrontal cortex and striatum and demonstrates neurophysiological correlates

of drug cue-evoked pupil responses and interindividual variability in those responses. We also used those data in a novel way to assess hallmarks of neural criticality and system complexity in the cortex and striatum. This in itself represents another first in the fields of self-organized criticality and addiction.

The first two chapters of this dissertation describe the creation of a protocol for head-fixed high-density recording of neurons in multiple reward circuitry hubs in mice that exhibit a conditioned response to cocaine cues. We first showed that head-fixed mice can exhibit conditioned place preference for an odor context that was associated with cocaine injections in a virtual reality paradigm, indicative of a learned association. We also found that the response was quite variable between animals and rather than remaining in place, many animals tended to run when experiencing the drug context. Subsequently, we showed that in response to novel or drug-associated odors briefly presented to them, mice exhibit a range of locomotor responses, and neural firing may have encoded a representation of the cue or the locomotor response.

The last two chapters demonstrate the use of a 512-channel electrode array for recording the cortex and striatum simultaneously in conjunction with pupillometry and cocaine conditioning. Pupillometry allowed us to assess the CR and have a behavioral readout that does not interfere with interpretation of physiology as well as track interindividual variability in cue responsiveness. We found that frontostriatal circuit dynamics correlate with cocaine cue-evoked behavioral arousal during early abstinence in mice. Small amounts of cortical pyramidal neuron hyperactivity, rather than hypoactivity during quiescence, was also observed. We then describe several aspects of universal critical dynamics and complexity in our datasets and how they relate to interindividual variability in our pupillary data and hyperactivity. We show that cocaine treatment enhances critical tuning and complexity in the cortex, while decoupling both phenomena in cortex and striatum, showing that there are real quantitative effects of drug usage on critical neural networks and system state variables.

The dissertation of Wesley Charles Smith is approved.

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Peyman Golshani

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DEDICATION

This dissertation for everyone who's wondered about the brain and why we are the way we are. This is for my family, that's been stricken with mental illness, and everyone around the world who carries that burden. This is for those addicts who I've known who've even stole my guitars to feed their addiction, and those that were recovering that we shared a musical bond with. This is for my deceased friend, Joe Herron, whose suicide was preventable, if we only had better options in treating brain disorders other than pharmacological agents that mimic methamphetamine and massively mess with the brain's reward system. This is for my wife, whose grace and wisdom enabled me to get this far. Thank you. From now, I'll build on this work and persevere to make the world a better place with better knowledge of how we know, feel, and think.

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Chapter 3, aside from minor formatting changes, is a version of the material as it appears in “Frontostriatal circuit dynamics correlate with cocaine cue-evoked behavioral arousal during early abstinence. *eNeuro.* 16 June 2016, 0105-16.2016. Wesley C. Smith, Matthew H. Rosenberg, Leslie D. Claar, Victoria Chang, Sagar N. Shah, Wendy M. Walwyn, Christopher J. Evans and Sotiris C. Masmanidis (2016).” The dissertation author was the primary investigator and author of this paper. Wesley Smith, Wendy Walwyn, Christopher Evans, and Sotiris Masmanidis designed research. Wesley Smith, Matthew Rosenberg, Leslie Claar, Victoria Chang, and Sagar Shah performed research. Wesley Smith and Sotiris Masmanidis analyzed the data and wrote the paper. All authors approved the final version of the manuscript.

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Andrew J. Weitz*, Zhongnan Fang*, Hyun Joo Lee*, Robert S. Fisher*, **Wesley C. Smith***, ManKin Choy, Jia Liu, Peter Lin, Matthew Rosenberg, & Jin Hyung Lee. “Optogenetic fMRI reveals distinct, frequency-dependent networks recruited by dorsal and intermediate hippocampus stimulations”. *Neuroimage*, February 15, 2015

Sunny Nigam, Masanori Shimono, Shinya Ito, Fang-Chin Yeh, Nicholas Timme, Maxym Myroshnychenko, Christopher Lapish, Zachary Tosi, Paweł Hottowy, **Wesley C. Smith**, Sotiris Masmanidis, Alan Litke, Olaf Sporns, & John Beggs. "Rich-club organization in effective connectivity among cortical neurons". *The Journal of Neuroscience*, January 20, 2016

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2015 Society for Neuroscience meeting in Chicago – "Large-scale multi-regional neural recordings reveal altered frontostriatal network dynamics following cocaine administration". **W.C. Smith**, L. Claar, M. Rosenberg, V. Chang, N. Chong, S. Shah, and S. Masmanidis

10th Annual Dynamics of Neural Microcircuits Symposium 2016 – "Frontostriatal circuit dynamics correlate with cocaine cue-evoked behavioral arousal during early abstinence" (poster). **W.C. Smith**, M. Rosenberg, L. Claar, V. Chang, S. Shah, W. Walwyn, C.Evans, and S. Masmanidis

INTRODUCTION

The basal ganglia, a chief network of subcortical reward system nuclei in the brain, are understood to encode activity correlates of reward prediction, movement initiation and goal-oriented behavior, as well as attribution of salience and value to actions and stimuli (Schultz, 1998; Everitt and Robbins, 2005; Belin et al., 2009). The elements of the basal ganglia referred to as the dorsal and ventral striatum in mice comprise the largest volume of the basal ganglia and contain mostly inhibitory neurons: The GABAergic medium spiny neurons (MSNs), fast spiking inhibitory interneurons (FSIs), and tonically active neurons (TANs) that are cholinergic (Prensa et al., 2003; Gerfen, 2004; Voorn et al., 2004; Berke, 2011).

MSNs are mostly seen to directly project to a smaller set of nuclei, the segments of the globus pallidus, whose projections are afferent to the thalamus, a chief input to the cortex (Albin et al., 1989). When the striatum is removed from a recently deceased mouse and electrophysiologically studied in a slice preparation, despite the numerous cells in it, very little spontaneous activity is observed (Koos and Tepper, 1999; Gage et al., 2010), lending credence to a fundamental concept in neurobiology: neurons must be anatomically connected to each other to communicate functionally as a system, or a so-called circuit.

Early experiments with stimulation of the medial forebrain bundle (MFB) fiber tract in humans and murine models showed that inputs into the striatum can drive appetitive responses and intracranial self-stimulation (Nieuwenhuys et al., 1982), thus demonstrating the necessity of a circuit to these behaviors early on. *Ex vivo* preparations sever these circuits, whose excitatory glutamatergic inputs from the cortex, ventral hippocampus, and lateral amygdala normally aid in the encoding of survival responses (Marek et al., 2013) and obtaining of natural rewards (Lansink et al., 2009). More recent experiments have shown that optogenetic, cell-type and pathway-specific, stimulation of said inputs can drive appetitive responses (Britt et al., 2012). Similarly, dopaminergic inputs that directly encode a reward-prediction error feed into the

striatum, which interacts with the aforementioned excitatory inputs (Schultz, 2016). Importantly, the release of this dopamine is what is predominately affected by drugs of abuse (Pulvirenti and Koob, 1990; Wise and Hoffman, 1992), and artificial stimulation of this pathway optogenetically also drives appetitive responses (Tsai et al., 2009).

Considering this system of interconnected brain regions, a memory beneficial for survival can be constructed through both explicit contextual and environmental information from the hippocampus and neocortex, as well as implicit information such as internal state or affective tone and habitual responses (Rossato et al., 2009; Luo et al., 2011). These simultaneously interacting regions demonstrate synaptic plasticity and facilitate the creation of long-term memories or “engrams”, the neurobiological substrates of a memory, purely through activity patterns and biochemical signaling. In the striatum, rewarding information can become more efficiently processed through altered synaptic weighting and long-term physiological output, the evidence of which there is a surfeit of (Tremblay et al., 1998; Costa et al., 2004; Xiong et al., 2015). Through such a memory, animals recall an action plan appropriate for each context they might come across (Cai et al., 2016; Josselyn et al., 2017).

Several models of addiction-related behaviors are used to understand how addiction can manifest in such actions (Koob, 2014; Farrell et al., 2018). Broadly, an interaction of time, context, individual internal state, and manner of drug ingestion can yield a behavior with a quantifiable dimension to assess how much an animal will work for a drug or how much an animal prefers a stimulus associated with a drug. A non-contingent behavioral protocol usually involves giving an animal a drug such that they passively receive it without effort. Typically, this is associated with an external context or cue such that the cue, when presented later, elicits a cue-motivated response or a conditioned response (CR). The CR may simply be movement or orientation towards the cue, or as much as attempting to interact with the cue as it would a natural reward such as food (attempting to eat it) if the associated reward were food, as in

autoshaping (Brown and Jenkins, 1968). Depending on the protocol, a non-contingent or Pavlovian response may be elicited by allowing animals to choose a context to remain in during the absence of drugs, if the choice is between a context or location that was associated with drugs and another that was not; this is called conditioned place preference (CPP)(Koob, 2014). If however the animal is trained to work for an intravenous drug injection via a lever press, nose poke, entry into a certain environment, or other effort, this is a contingent administration protocol (Markou et al., 1999). Testing for continued effort or perseverance of such responses in spite of increasing effort (scheduling) or adverse outcomes provides a metric through which craving or attributed value can be measured. Since addiction plays upon the previously described reward circuitry, the behaviors utilized involve motor actions, planning, repetition, and internal value assessments.

Of note is that in the addiction field, there is no consensus on which method of animal protocol best recapitulates the varying phenomena of drug taking, seeking, and relapse in human patients addicted to drugs of abuse (Kalivas, 2009). Typically, when humans take drugs, they usually take a single bolus or brief but potent dosage of drug at a concentration that provides psychoactive effect. Thus, this has elements of contingent administration (Epstein et al., 2006), but the effects of the drug would be most similar to experimental non-contingent administrations that typically have larger doses (Tzschentke, 2007). Animal studies that provide objectively aversive consequences to drug schedules of reinforcement such as foot shocks cannot replicate the nuanced negative effects that humans experience, such as the slow decline of financial stability and personal health, loss of relationships, and other negative outcomes. However, aversive conditions like foot shocks upon contingent responses for drugs (Kasanetz et al., 2010; Chen et al., 2013) need to be present to be able to measure drive to take drugs at all, and are a natural limitation of working with murine models. Finally, when asking human patients if they like a drug or simply want it to stave off withdrawal, as is a consequence of negative

reinforcement, the answer can be measured simply (Potgieter et al., 1999). In murine models, assessing liking versus wanting drugs is difficult, and disentangling the circuitry that encompasses both or either of these seeking-related behaviors is its own challenge (Berridge, 2009; Wassum et al., 2009). Due to this, each protocol for using a drug in an experimental context requires sound reasons for experimental setup and choice of behavioral readout such that false conclusions are not reached about animal motivation or internal state. The method of administration also reflects a point about limitations of experimental design and caveats of conclusions drawn.

Neural correlates of reward prediction, movement initiation and goal-oriented behavior, as well as attribution of salience to reward-predictive cues are thought to be supplanted by maladaptive or compensatory (Kalivas, 2009; Luscher and Malenka, 2011) changes inside the basal ganglia and its connected regions during addiction, particularly with cocaine, and these are all based on the aforementioned memory circuitry. Since most drugs of abuse act either to modulate released dopamine in the basal ganglia/ventral striatum or the activity of the source of dopamine in the midbrain's ventral tegmental area (VTA), one of the origins of the MFB (Adinoff, 2004; Pierce and Kumaresan, 2006), most investigators have focused energy into uncovering cellular correlates of addiction in those regions separately, due to technical limitations.

Through such reductionist efforts, it has been found that the infralimbic (IL) and prelimbic (PrL) cortices within the medial prefrontal cortex (mPFC) have a role in drug-seeking behavior and reinstatement of seeking after extinction of seeking behavior (Volkow et al., 2005; Peters et al., 2008; Chen et al., 2013; Ma et al., 2014). This may be observed through drug-induced or optogenetically induced prefrontal cortical hypoactivity. These regions strongly project to the aforementioned ventral striatum, albeit with the core of the accumbens receiving primarily prelimbic projections and the shell receiving infralimbic afferents (Vertes, 2004; Voorn et al., 2004). Here, hypoactivity refers to an *in vivo* and ex vivo observation (Trantham et al., 2002) of

reduced pyramidal neuron membrane bistability, increased threshold to spiking, and lower spontaneous firing rate (Chen et al., 2013), with potential human correlates as seen in functional imaging (Goldstein and Volkow, 2011). It is hypothesized that this resting hypoactivity is sufficient to induce relapse and increase drug-seeking, suggesting that resting dysfunction of the mPFC reduces glutamatergic control of the striatum. Concomitantly, cortical dendritic morphology changes markedly over weeks in the incubation period of addiction (Munoz-Cuevas et al., 2013), while silent synapses in the cortical and amygdala inputs to the striatum are formed and mature (Lee et al., 2013; Ma et al., 2014). This period corresponds to a time at which animals are not allowed access to drugs, but their subjective craving responses increase after a withdrawal state that includes opponent processes that may be related to stress (Koob et al., 2014). These changes into the striatum coincide with anaplastic changes in the striatum with a reduction of LTD processes (Martin et al., 2006; Kasanetz et al., 2010; Kasanetz et al., 2013) and an alteration in homeostasis for extrasynaptic spill-over glutamate (Kalivas, 2009; Trantham-Davidson et al., 2012). In addition to basal changes of physiology, cells in ventral hippocampus, mPFC and the ventral striatum become responsive to drug-predictive cues (Carelli et al., 1993; Ghitza et al., 2003; Pennartz et al., 2011).

As previously mentioned, brain studies in human patients addicted to drugs of abuse have shown a variety of large-scale effects through PET and fMRI imaging. In particular, drug-predictive cues shown to patients increase metabolic signals in PFC and distributed limbic structures, the magnitude of which correlates to drug craving (Grant et al., 1996). Cocaine cue-evoked measures of functional connectivity between the cortex and striatum has also been shown to be altered or dysregulated between the cortex and and striatum in human addicts (Wilcox et al., 2011; Hu et al., 2015), which also may be similar to pathological gambling or behavioral addictions (Koehler et al., 2013). This may be related to how PET studies in humans and primates have shown that an inverse relationship is observed between striatal D2 dopamine

receptor availability and frontal metabolism and reward susceptibility (Volkow et al., 1993; Volkow et al., 1999; Nader et al., 2006; Dalley et al., 2007). Structurally, deficits in right inferior frontal gyrus grey matter volume exist and total volume of frontal grey in addicts correlates to emotional regulation ability and inversely correlates to drug craving (Moreno-Lopez et al., 2012).

Interestingly, only a subset of those who try drugs of abuse such as cocaine become addicted to them; in the case of cocaine, only around 15-16% of those who first try it become addicted within the same decade, and around 15–19% of recent cocaine users are dependent (Wagner and Anthony, 2002; O'Brien and Anthony, 2005). These figures depend strongly on many factors including age, gender, socioeconomic status, and method and context of self-administration, with certain controlled real-world environments leading to less self-harm, albeit no absence of it altogether (Cohen and Sas, 1994). In murine models that closely resemble addiction, a similar observation has been made when drawing a distinction between rats that that are resistant to footshocks when actively responding for self-administered cocaine infusions intravenously and those that are not resistant. Roughly 15-19% of rats in these breakpoint/footshock studies exhibited multiple criteria for addiction-like behaviors, a highly consistent result with human studies (Deroche-Gamonet et al., 2004; Kasanetz et al., 2010). Additionally, research suggests that the stress of footshocks even supports the acquisition of drug seeking, which may be due to poor impulse control (McFarland et al., 2004; Belin et al., 2008; Buffalari and See, 2009; Economidou et al., 2009). The source of this variation in response to drugs could reside in aberrant plasticity, or anaplasticity and resistance to LTD-based synaptic scaling. Since LTD in the striatum is based on cortical glutamatergic input, a large-scale method to assess all neuronal firing in a section of cortical and striatal tissue may yield insight on how there is differential encoding of information in animals that exhibit more hallmarks of drug addiction.

Larger scale methods to study addiction in murine models have shown consistent evidence that many neurons actively encode context or environmental stimuli associated with drug treatment, and their specific ablation can hinder behavioral indications of drug-craving (Cruz et al., 2013). In rats that are incubating cocaine craving, behavioral tasks show poor measures of set-shifting and reversal learning, markers of cognitive flexibility (McCracken and Grace, 2013), consistent with results in humans and primates that show cocaine usage can impair cognition and reversal behaviors (Jentsch et al., 2002; Goldstein et al., 2004). When multiregion electrophysiological recordings were performed these anesthetized craving rats, they showed lowered spontaneous LFP power across multiple basal ganglia structures including the prefrontal cortex, but increased evoked LFP oscillation power due to basolateral amygdala stimulation, indicating that the circuits of striatal inputs at rest are functionally changed after drug treatment and withdrawal.

Reduced preparations and reductionist methods to observe exact ion channel or gene function in a particular context is often thought to determine causality within a neurobiological problem or framework, for example, one aspect at one timescale in one behavioral model of addiction. In actuality, the determination of necessary or sufficient conditions to establish disease or function does not necessarily prove causality, especially considering translational work from murine systems to primates and humans as well as incomplete understandings of preparations or experimental conditions. At best, highly-specific experimental designs to probe functionality in addiction, a complicated systems-level process, are incomplete yet strong correlative tests. A chief limitation of cellular and physiological work in murine models of addiction has been the reductionist approach of sampling from small numbers of cells or using *ex vivo* tissue, neither of which can approximate a real network of coordinated neurons *in vivo*, which is now the impetus for a more concerted research effort in understanding the functional neural connectome (Alivisatos et al., 2012). Alternatively, neuroimaging of human addicts has

revealed the aforementioned insights into broad changes associated with addiction, with the drawbacks of low temporal resolution, a lack of experimental control, and an inability to infer microcircuit dynamics from BOLD signals (Logothetis, 2008).

Thus, there exists a fundamental scarcity of data regarding large-scale neural dynamics in the context of addiction, particularly in the corticostriatal path. Included in this, there have been no studies thus far to address the large-scale manifestation or emergent properties of hypoactive cells in the cortex after cocaine treatment, and no studies that have been able to test interregional differences in cortical responses to drug cues or quiescent cortical rates, let alone interregional dynamics between cortex and the striatum at the single-cell level. How these related to interindividual variability in response to drugs and their cues is also unknown. Current large-scale research in the addiction still cannot probe deep brain tissue like the striatum or deep mPFC *in vivo* and assess functional connectivity of hundreds of neurons due to the optical limitations of 2- or 3-photon imaging (Horton et al., 2013; Ouzounov et al., 2017), nor can those methods assess systems-state measures like LFP. Even more modern techniques like microendoscopy with GRIN lenses also offer chronic imaging and deep structure imaging, but with their small field of view, can only image hundreds of cells in a particular brain region that has a densely active laminar structure, like the cerebellum; at other times, the resolution is of dozens of cells at best (Ghosh et al., 2011). Although this technique apparently does not interfere with general motor behaviors by implanting miniaturized microscopes into brain tissue, issues remain with brain tissue aspiration, which is a model of brain injury, the extent of which depends on how much tissue is aspirated and the size of the inserted lens (Lee et al., 2016). This technique also requires transgenic mice with GECIs (genetically-encoded calcium indicators) that are often too slow to resolve single spikes, let alone field potentials, even with faster ones becoming available every year (Thestrup et al., 2014). Existing high-throughput recording techniques, even in their most advanced forms, have yet to be able to record multiple

distant brain regions with more than 100 channels while retaining meaningful single unit data (Mendoza et al., 2016; Xie et al., 2016), and are limited to local circuits even at 512 channels (Berenyi et al., 2014). Thus, there is a need to establish an electrophysiological view of the deep cortex and striatum working together in addiction, so as to better understand the functional correlates and single unit underpinnings of addiction and relapse to drug-paired cues, as well as large-scale correlates of hypoactivity/hypofrontality. Ultimately, by understanding how more neurons and more brain regions serve to build representations of actions and outcomes in a normal or addicted condition, translational research, reductionist models, and basic understanding of brain function can be improved upon.

This dissertation aims to address the dearth of systems-scale *in vivo* addiction research by recording in vivo hundreds of neurons in the corticostriatal path from head-fixed mice after cocaine conditioning. First, we show that head-fixed mice can undergo one type of behavioral conditioning with cocaine and exhibit a type of conditioned place preference (CPP) in virtual reality (VR), with odors delineating space along linear quadrants that are navigable via a uniaxial treadmill. This represents a novel conditioning environment since the mice are head-fixed, moving, and behaving with multiple cues present, and also enables usage of acute, customized, high-density electrode arrays (Du et al., 2011; Bakhurin et al., 2016). We then learned that the behavioral response to the quadrant paired with cocaine was variable, so we modified our behavioral test to yield an autoshaped response or conditioned response (CR) of movement evoked by brief presentations drug-paired odorant cues only.

We built an electrode array to record from more connected parts of cortex and ventral striatum (VS) simultaneously, based on literature reviewed (Ding et al., 2001; Gabbott et al., 2005). Behavior from this modified protocol was also highly variable and not normally distributed, and neural firing could encode both drug cues and running responses, which was ambiguous. We subsequently describe the modification of our protocol to utilize pupillometry, an autonomic

marker of arousal, which is conserved as a response to drug cues in humans (Rosse et al., 1995), and which can be measured to have noticeable fluctuations even during locomotor and general behavioral quiescence (Reimer et al., 2014).

A novel electrode array was constructed according to piloted Fluorogold (FG) tracer experiments, and we designed the array to record from the core of the accumbens and from PrL and IL cortices. Using our new protocol, we briefly presented odors that were associated with cocaine or saline in the absence of injections and assessed pupillary responses. We found a conditioned autonomic response to the cocaine paired cue in the form of increased dilation in the absence of locomotion. Per animal, this was correlated to corticostriatal inhibition and increased beta-gamma (25-45 Hz) LFP coherence between both regions. Irrespective of causality or assessment of direct monosynaptic control of the striatum or occulomotor regions including the Edinger-Westphal nucleus or locus coeruleus, corticostriatal network activity showed a high correlation to our behavioral measure in the absence of confounding locomotion, and the task itself was a rudimentary recall task. This change in processing cues and its ties to multiple connected brain regions suggests that rather than quiescent inactivation of control from the cortex, aberrant active output suppression and enhanced corticostriatal processing may be a deciding factor in addiction and interindividual variability.

Surprisingly, our experiments didn't yield quiescent hypoactivity in the cortex. Rather, we found near significant hyperactivity and followed up this result by writing and using custom neural avalanche detection scripts to discover that this hyperactivity may manifest in the form of increased cortical criticality at rest (Beggs and Plenz, 2003), suggesting a shift in resting dynamics towards a more critical brain state in drug-treated mice. This also was correlated to interindividual variability in cue responses, suggesting that critical neural dynamics have a role in learning and addiction, and bear further research, as it has recently been found that techniques to aid in setting the brain to a critical state like mindful meditation also coincide with

proper self-care and relapse prevention (Hankey and Shetkar, 2016). Finally, we found enhanced complexity in the cortex, but not striatum, in drug treated mice at rest. Interestingly, both regions had complexity decoupled from critical tuning in the drug treated condition, whereas in the control condition, criticality was highly correlated to complexity in both regions, which may be related to our aberrant LFP observation at rest. In all, our data proved to be quite amenable to neural criticality analyses, which insofar to our knowledge, has been relegated to EEG, fMRI, slice culture, and shallow brain recordings both with low-density electrodes or slower fluorescent activity reporters. This novel combination should yield great improvements in studies of criticality on the mammalian brain, as deep brain regions are now accessible with our high-density electrode arrays.

Examining conditioned place preference in response to cocaine conditioning in virtual reality (VR)

Cocaine is a schedule II controlled substance and a psychostimulant with certain therapeutic uses as a local anesthetic (Ruetsch et al., 2001). However, its non-medical use as a psychostimulant drug of abuse constitutes the vast majority of its use, with yearly black market sales up to $80b (United Nations Office on Drugs and Crime., 2014), surpassing the net worth of the top pharmaceutical company in the world (Knewitz, 2018). What makes using cocaine so profoundly rewarding and euphoric such that illicit usage remains so great is still an active subject of research. In addition to the rewarding effects of cocaine, the opponent effects and negative-reinforcement aspect of psychostimulant addiction are a chief factor in perseverance of usage in human addicts, and understanding how they mediate the course of addiction and seeking is of prime importance (George and Koob, 2017).

The principle effect of cocaine is as a dopamine transporter blocker, such that dopamine remains signaling within the synaptic cleft rather than internalized back into the terminals of

dopaminergic (DA+) neurons (Koob and Nestler, 1997). The synapses most greatly affected are within the striatum, prefrontal cortex, and in midbrain dopamine autosynapses. As previously mentioned, dopamine generally serves to motivate behavior or drive expression of a behavior with rewarding outcomes, although dopamine release is also tied to aversive events and learning of outcomes through reward prediction error. Interestingly, dosage amount of a drug is not enough to drive behaviors that are reward-related, rather, the method of ingestion dictates how bioavailable the drug is, and how rapid and strong a drug's effects are manifested (Barnett et al., 1981). In the case of insufflations or intravenous injection, cocaine can be more than twice as bioavailable than by ingestion. Using such cocaine injections in awake-behaving mice, we wanted to establish a systems-physiology model of cocaine-experienced mice. Given that we use custom electrophysiolgical tools in the lab that require head-fixing (Du et al., 2011), we first needed to show that addiction-related behaviors could be observed with our canonical mouse-on-a-ball setup.

In chapter one, we demonstrate that a subcutaneous (SQ), rather than intravenous, injection of cocaine solution in head-fixed mice paired with a context in a virtual environment reveals a CR when tested in the absence of drugs. In our case, the CR is CPP with a longer time spent in a drug-paired VR quadrant than other non-paired quadrants. First, we habituated mice to being head-fixed on a uniaxial treadmill. Following that, mice were allowed to experience a linear recursive virtual maze while on the treadmill that contained four discrete constant odor and visual zones that corresponded to 42.5 cm of space on the track. On the following day, animals were placed in only one of the previous contexts while head-fixed for 30 minutes and received an associated saline injection. This alternated with a different odor/visual zone consistently paired with a cocaine injection (20 mg / kg) on the subsequent day, such that three total saline injections and three total cocaine injections were given. Animals simply walked through the recursive VR environment as they experienced the odor and visual stimuli paired

with the injection effects. We demonstrate that on only cocaine-conditioning days, mice undergo classic psychomotor sensitization, in that mice that are given repeated cocaine doses exhibit more and more locomotor and behavioral effects (Lakoski et al., 1992). After six days of such training, head-fixed mice were exposed to the original recursive VR environments that included both the saline and cocaine paired environments separated by a neutral environment in a linear fashion. During virtual environment exploration, animals paused in the drug-paired environment, and the duration of that pause was measured as CPP. Animals trained on cocaine spent more time in the drug paired context compared to mice that only ever received saline injections in both contexts. However, this difference failed to reach statistical significance during multiple comparison testing.

Interestingly, more than half of the mice tested in this way exhibited hyperlocomotion, or an alternate appetitive CR in response to the drug-paired odor context (Michel and Tirelli, 2002). This sort of placebo effect of context is a known confound of CPP, which itself is known to have problems with it as a form of autoshaping, rather than a test of preference to remain sedentary (Newlin, 1992). Thus, to ensure behavioral results are categorically the same with variance that allows for detectable differences in treated and untreated groups, we changed the protocol of the experiment so that we could detect cue-evoked locomotion averaged from multiple trials of cue presentations, rather than allow mice to experience odors as they move through the virtual track.

Cocaine conditioning results in variable Pavlovian approach behaviors in head-fixed mice

In chapter two, we demonstrate the modified version of the previous experiment that utilized a similar conditioning protocol, but a changed probe or test protocol. In these experiments, mice in the habituation phases were trained to become more proficient with

moving on the treadmill, and the probe no longer required that the animals navigate through a neutral or saline-paired odor/visual zone to reach a previously drug-paired odor/visual zone. Rather, on test days mice were passively and randomly given odors that were paired with saline, cocaine, or unpaired , with a ~20s inter-trial interval (ITI) and we assessed ball locomotion as a CR and test for appetitive motion and by proxy, recall of the drug experience. These drug-treated or saline-only animals still were probed in the absence of any injections and after 24 hours of their last injection. A small cohort of animals was also trained on the task but incubated cocaine craving for 21 days after their final conditioning day, to assess behavioral and large-scale neurophysiological effects of incubation. Drug doses were reduced to 15 mg/kg from 20, and we increased the conditioning days from 3 to 4 per treatment type, for a total of 8 days.

We also describe the usage of a novel 512 channel electrode array for the recording of multiple brain regions within the cortex and striatum simultaneously, as described earlier. We provide preliminary firing data and demonstrate robust, putatively cue-related firing properties of the neurons. However, connecting these results to behavior proved difficult, as mice in our experiment seemed split on their locomotor response to the odors, much like the pseudo-CPP protocol we previously ran. We found that 7 mice that were trained on the drugs did not run appetitively in response to the drug-paired cue, while 4 that did respond, robustly ran in response to only the drug-paired odor. These results were not significant, and ultimately, variability in running responses and an inability to accurately tie neuronal firing to cue-responses without associating them to correlated motor output (Cohen and Kohn, 2011) led us to a third and final behavioral metric used to assess recall of the drug-paired cue: pupillometry. Pupillometry would allow us to record a behavioral response in the absence of running, as pupils dilate during varying levels of arousal (Aston-Jones and Cohen, 2005; Vinck et al., 2015; Joshi et al., 2016), and if we did not need mice to run, we would not have to train them to run on the ball during habituation, and quiescence would be preferable. We hypothesized that this

would enable stable baseline dilation with a cue-elicited increase in response to the drug-paired cue. Prior to these behavioral experiments, we ran a set of FG injection pilot experiments to better pick recording targets in a smaller region of cortex and VS. This served to fine-tune the design of the 512 channel array to reduce issues of multiple comparisons in brain regions assessed.

Frontostriatal circuit dynamics correlate with cocaine cue-evoked behavioral arousal during early abstinence

In chapter three, we assessed the utility of pupillometry as a marker for behavioral arousal or drug cue recall as previously described, along with concomitant awake behaving recordings using the new probe design. We found consistent psychomotor sensitization during the conditioning period for n = 10 drug treated mice and no such effect for the saline-only controls. This effect was similar when looking at pupillometry data, albeit with a notable ceiling effect. We then found that indeed, drug-experienced mice have an increased change in pupil dilation in response to drug-paired cues vs. saline-paired cues, and this was generally in the absence of locomotion on the treadmill. This response was quite variable among drug treated animals, however, which is consistent with human addicts having different subjective experiences and responses to drugs and drug cues (Brousse et al., 2010; Nasser et al., 2015; Singer et al., 2016).

Quiescent firing rates yielded no significant changes in any cell population examined using a compartmental analysis after correcting for multiple comparisons, albeit with a slight trend towards hyperactivity in pyramidal cells rather than hypoactivity within the electrode group corresponding to IL. Later we postulate that such an effect may be due to a bias towards relatively active neurons in the spike sorting methods or a real neurobiological effect of critical system dynamics as examined in the following chapter. Both saline-paired and cocaine-paired

cues yielded no significant differences in proportions of excited or inhibited neuron populations in both cortex and striatum within 24 hours of the last drug dose, which may simply be due to a lack of time to enable the circuit-level changes that bring about cue-selectivity in the incubation and craving responses, or that the regions probed don't contain cue-discriminatory neurons.

Although pupil responses were variable and population responses to cues per animal were equivocal, we found that the pupil responses to the drug-cue in each drug-treated animal were highly and significantly correlated to the proportion of inhibition of projection neurons in both regions. This suggests that a possible mechanism for understanding inter-individual variability in drug response lies in suppression of the corticostriatal circuit, and perhaps brings about a transition to an addicted state. This observation was only true for the drug-treated animals and inhibition of projection cells in response to the drug cue, as the saline-paired cue elicited no such correlation, largely also in part due to drug-treated mice having no trend in response to such a neutral cue.

Finally, we took advantage of our electrode array allowing us to record inter- and intraregional LFPs to examine if synchrony between the cortex and striatum has been perturbed by drug treatments. Indeed, the spontaneous beta-gamma band of LFPs during quiescence had an increase in coherence (amplitude and synchrony). Drug cues also induced brief changes in low gamma band coherence, and we found that the magnitude of this increase was highly and significantly correlated to the pupillary response in each drug-treated animal. The magnitude of the coherence change for the saline paired cue was uncorrelated to pupillary responses to the saline paired cue. This suggests that corticostriatal coherence at rest has been altered, and we postulate that the population level inhibition previously observed may be working to enhance this change in synchrony in the corticostriatal circuit by removing neural noise to better enable cue discrimination.

Hallmarks of neural criticality and universality in a model of early abstinence from cocaine

In chapter four, we hypothesize that since our electrophysiological data have a sufficient recording duration and number of channels, we should be able to observe quiescent changes in firing properties of cells, contrary to the results shown in chapter three, given that we observed a trend towards hyperactivity in pyramidal neurons and spontaneous corticostriatal LFPs were altered. We developed a new set of analysis tools to look at firing rates across the electrode array in terms of neural avalanches (Beggs and Plenz, 2003, 2004; Beggs and Timme, 2012) and assess critical neural dynamics (Beggs, 2008; Timme et al., 2016; Cocchi et al., 2017). We had to first make assumptions about how the electrode array recording within in a section of cortical or striatal tissue in our experiments would record similar dynamics to a flat electrode array that records from organotypic slices (Friedman et al., 2012). We then demonstrated multiple important similarities between our results with identifying neural avalanches and previous results from established researchers in the field of critical neural dynamics (Boonstra et al., 2013). We first observed that our i*n vivo* avalanches in general follow the same shape function as in organotypic slice data and have a “crackling noise” property, then that their duration and magnitude distributions follow a power law (are scale-free) with exponents close to a negative golden ratio, and finally that our observed avalanches can exhibit fractal patterns when multiple duration groups are plotted together as shape collapses (Sethna and Dahmen, 2004; Friedman et al., 2012), a key sign of universal scaling and scale-free data. We then compared spontaneous neural avalanche collapse data between our drug-free mice and drug-treated mice and observed greater tuning towards criticality and complexity in our drug-treated mouse data by using two mathematical methods. This may underlie aberrant cognitive processes that bias them towards drug-related thoughts or make them pay more attention to drug-related cues. Additionally, we found that the degree of universality is correlated to

behavioral responses, but not complexity, another measure of interindividual variability. One alternate explanation we cannot rule out is if merely the association of being within the same drug treatment room is enough to instigate a more critical-like mental state in drug treated mice during quiescence, independent of potential baseline-changing effects of cocaine. Naturally, however, criticality is thought to emerge to enable flexibility in responses to stimuli in a changing world, and alterations in criticality as discussed here may work similarly to how arousal and drowsiness affects critical dynamics and mental states in humans, peaking during drowsiness or in a state like day-dreaming or the default mode (Deco et al., 2011; Hearne et al., 2015; Breakspear, 2017; Hahn et al., 2017). Even if it were the case that the contextual trigger alone was driving a more critical brain state, it would be of interest to study how learning alters network dynamics in such a sweeping fashion, especially relative to controls that showed no such learned associations. However, as assessing critical dynamics *in vivo* as part of understanding quantitative effects of brain states is a new field, care must be taken in drawing further conclusions. More experiments with different drugs of abuse in various contexts familiar and unfamiliar to test subjects are needed to ascertain if indeed drugs of abuse modulate criticality and if criticality poses an essential aspect to proper brain function, learning and memory, or addiction and vulnerability to relapse.

Conclusions

In conclusion, we have shown that cocaine conditioning can occur in head-fixed mice via a novel conditioned measure of arousal. These results showed that large-scale inhibition at the population firing and resting-state LFP levels were highly correlated to behavioral responses to drug cues, indicating a fronto-striatal network that undergoes active inhibition and enhanced synchrony selectively after drug use only. Finally, we also show changes in neural avalanches and critical neural dynamics in quiescent behavior periods in drug-experienced mice that may

underlie these large-scale population measures. Through understanding the large-scale neural encoding of drug cues and how drugs alter the baseline physiology of neural networks, we may be able to design better pharmacological and behavioral interventions to change the course of drug addiction and reduce compulsive drug seeking in patients addicted to substances of abuse. Our novel toolbox to assess neural criticality represents another method to pick apart large datasets and find patterns where the system appears almost insurmountably dense. Ideally, it serves to describe how complex systems interact and is applicable beyond neuroscience.

CHAPTER 1

EXAMINING CONDITIONED PLACE PREFERENCE IN RESPONSE TO COCAINE CONDITIONING IN VIRTUAL REALITY (VR)

Abstract

The exact nature of why cocaine is highly rewarding to those who take it is unknown and such effects could be mediated by deregulated dopaminergic and glutamatergic neurotransmission, as well as other monoaminergic reuptake mechanisms. A key issue is that both types of neurotransmission work on multiple interacting brain systems, and while individual regions have been amenable to electrophysiological recordings, as yet in the context of addiction or substance abuse, awake-behaving recordings across multiple regions have not been performed in murine models. Thus, the nature of the interaction between the striatum and its glutamatergic inputs when an animal experiences drugs or drug cues and how they are encoded remains a “black box”. The study first attempts to create a head-fixed protocol that allows multi-region high-density silicon array electrophysiology and behavior measurement in the context of a transition to addiction. To demonstrate that mice can experience the posited euphoric effects of cocaine before any electrophysiology, we designed and piloted a conditioned place preference protocol in VR. We found that a medium-high dose of SQ cocaine (20 mg/kg) elicits psychomotor sensitization in 8 mice and when pooled, the results showed signs of CPP. However, only half of the mice tested revealed a strong preference, biasing the results towards significance. These results suggest that place preference in VR may be less amenable to study behavioral manifestations of cocaine association, and informed the experiments in the following chapters in selecting an alternative CR to measure and pair with electrophysiology.

Keywords: cocaine, head-fixed behavior, basal ganglia, conditioned place preference, drug conditioning, virtual reality

Introduction

Medicinal cocaine has a narrow usage as a local anesthetic due to its ability to block sodium channels and thus action potentials (Crumb and Clarkson, 1990). However, this usage is small compared to illicit usage of the drug for its psychoactive and euphoric properties, as cocaine is one of the most trafficked illegal drugs in the world (United Nations Office on Drugs and Crime., 2014). Cocaine's psychomotor and euphoric effects are well-documented (Wise, 1984; Wise and Bozarth, 1985; Nestler, 2005), and they are thought to be brought about in part through its monoamine transporter blocking actions. Briefly, subjects who take cocaine via insufflation, injection, or inhalation of vapors, experience exhilaration and enhanced energy, heightened alertness, hypersensitivity to certain sensory stimuli, increased irritability, and extreme or unreasonable distrust of others. As previously mentioned, there is up to a 17% chance of becoming a cocaine abuser upon trying it, with drug abuse being defined in the DSM-V as a relapsing disorder characterized by compulsive drug seeking and loss of control over intake despite ongoing adverse consequences (American Psychiatric Association, 2013)

Underpinning that 17% figure is a complex interplay of the genetics and epigenetics of the drug user along with environment and personal history (Sinha, 2009). The drive for the euphoria may be an initial urge to experience a release from stress, be it from peer pressure or personal stress brought on by economic or familial hardship; even the innate inability to suppress impulsive actions may be a stress response, to permit an action that a user might find relaxing or supportive (Economidou et al., 2009; Koob et al., 2014). Additionally, it has been observed that when rats are raised without social or environmental enrichment, they tend to choose opiate-laced water over regular water, indicating that seeking euphoria or altered states of consciousness may be born out of a variety of personal stressors, one of which is a lack of natural or physiological stimuli (Alexander et al., 1978). There are some caveats to these

studies (Petrie, 1996), but they supplement existing research on stress and addiction (George and Koob, 2017).

The euphoric itself appears to correspond to increased synaptic dopamine in the striatum which is a central idea of the “dopamine hypothesis of addiction” (Wise, 1984; Luscher, 2016). However, other drugs which don’t necessarily affect striatal dopamine immediately also result in euphoria, thus making other inputs to the basal ganglia loop and neurotransmitters key players in addiction and euphoria (Nutt et al., 2015). Here, when we refer to the basal ganglia loop, we are simplifying it to the outputs of the medial striatum that use dopaminergic inputs from the VTA and substantia nigra to influence responses in the lateral and dorsal aspects of the striatum itself, not only the cortico-striato-cortical loop of the canonical direct/indirect pathway (Belin and Everitt, 2008; Belin et al., 2009).

When drugs are difficult to obtain or the user abstains from drug usage, effects of withdrawal set in, which may be a greater reason for relapse than desire for euphoria. The effects of cocaine withdrawal are multiphasic and can include but are not limited to fatigue, depression, anxiety, dysphoria, and sleep issues (Koob et al., 1997; Walsh et al., 2009). Experimental literature suggests that over time, usage of drugs sets the brain into a state that produces a negative emotional state that drives persistent, relapsing drug seeking during abstinence (Koob and Le Moal, 2001). This may be related to the observation that D2 dopamine receptors are unavailable to bind DA in human addicts (Volkow et al., 1993; Volkow et al., 2001). The aforementioned outside elements of the basal ganglia loop that innervate the basal ganglia include the glutamatergic hippocampus, amygdala, and prefrontal cortical regions, which may underpin the long-term changes in physiology and behavior. Several markers of cocaine abuse and maladaptive plasticity can be observed physiologically in these regions, dependent on time course of usage and abstinence (Luscher, 2016).

The hippocampus, for example, is required for cue-induced cocaine craving and the acquisition and processing of cocaine memory engrams (Robbins et al., 2008; Trouche et al., 2016), while also bidirectionally synapsing with the VTA (partially through the striatum via the accumbens), PFC, and amygdala. Hippocampal neurogenesis is reduced during the transition to cocaine addiction, and inhibiting hippocampal neurogenesis increase drug seeking (Castilla-Ortega et al., 2016). Whole brain imaging has revealed that resting state functional connectivity in the hippocampus is changed in cocaine addicts, albeit in both directions, depending on the parameters and subjects within the study (Gu et al., 2010; Ma et al., 2010; Wilcox et al., 2011; Sutherland et al., 2012). Outside of the hippocampus, long term changes in silent synapse maturation and unsilencing during incubation of craving have been found within the amygdalostriatal path, along with similar effects within the frontostriatal path (Lee et al., 2013; Ma et al., 2014). Interestingly, prelimbic/infralimbic subdivisions in the cortex yield opposing mechanisms on seeking during craving. This may be because those mPFC subregions differentially encode cocaine-associated stimuli and differentially regulate incubation of craving by recruiting calcium-impermeable (core) and calcium permeable (shell) AMPARs into their associated striatal projections (Ma et al., 2014; West et al., 2014). Additionally, prefrontal hypoactivity and resistance to current injection after chronic cocaine exposure appears to be causally linked to drug seeking (Chen et al., 2013).

Since slice physiology preparations of the MSNs in the striatum lack intrinsic firing, it stands to reason that the excitatory afferents into the striatum are at least partly responsible for striatal output, are key players in patterning striatal activity *in vivo,* and likely play a role in aiding an animal's reward-learning in the context of natural survival. It is this patterning that may be going awry when observing the aberrant phenotypes previously mentioned. The hippocampus is crucially important for acquisition of contextual or declarative memories, as in, external information that is encoded by the brain in an engram or neurobiological substrate that may

include a pattern of activity (Eichenbaum et al., 2007). Engrams may be “stored” in such a pattern distributed across neurons and then recalled for the benefit of an organism’s survival. Declarative memory specifically refers to factual information or concepts derived from the environment as in semantic memory, or personal experiences, as in episodic memory. On the other hand, implicit memories, those that are used to perform a task without the conscious thought of performing that task (Voss and Paller, 2008), rely on non-hippocampal structures such as the amygdala, cortical regions, and striatum, as in procedural memory (Barnes et al., 2005).

It stands to reason that declarative memories of drug-associated cues or environmental stimuli would be fed into the striatum via patterned hippocampal activity, and could be potentially reinforced via altered neurotransmission after drug receipt. The output of the basal ganglia, canonically voluntary action or movement (Shin et al., 2018), may be primed to maintain a new state of homeostasis and aid in drug-seeking due to addiction withdrawal or desire for euphoria , drug- seeking and taking being a form of learned habit or implicit memory (Robbins et al., 2008; Goodman and Packard, 2017). Rather than maintain a coherent pattern of reasoned choices, as in weighing the pros and cons of continued drug taking, the user will lapse into seeking and taking drugs based on various external and internal cues, all based on implicit cognition (Stacy and Wiers, 2010). Driven by contextual inputs, the basal ganglia can provide behavioral outputs that are measurable indicators of addiction.

Although there are several ways to demonstrate (Koob, 2014) a recall of a drug associated cue via behavioral outputs , they are rooted in two common techniques used in lab settings to first administer drugs of abuse: The “non-contingent” protocols and the “contingent” protocols. Contingent refers to the administration of drug being contingent on the subject performing a task, as in operant conditioning, to receive a drug. This usually occurs in a fixed schedule during which a subject is tasked to learn the association between the action and the

reinforcer or drug experience – the fixed schedule refers to the amount of work vs. the iterations of reward receipt. That is, a fixed-ratio 5 reinforcement schedule means that for every fifth correct response, a reward will be administered. With drugs, this could be intravenous, subcutaneous, or through a variety of other methods. Testing for a drug memory in this case means assessing how perseverant a subject is in seeking drugs when put in the same context, including with predictive cues to initiate the operant task, but without the reinforcer. Frequently, time to extinguish the responses is measured, along with how quickly the subject may relapse back into responding after abstinence if a cue is present, and how much the subject responded without drugs, among other measures. In general, it is thought that contingent protocols best model aspects of how addicted individuals self-administer drugs and how behavior and neurophysiology changes due to that method (Epstein et al., 2006; Kalivas, 2009).

Non-contingent administration doesn't require a subject to perform a task to obtain a reward, rather, they are exposed to context that invariably predicts and provides some form of reward. To assess a memory of this association, conditioned place or context preference is frequently measured, although it is possible to measure a response as in autoshaping, which is a modified unconditioned response to a stimulus predictive of whatever unconditioned stimulus that has been provided (Brown and Jenkins, 1968; Steinhauer et al., 1977). To assess CPP in the absence of drugs or rewards, subjects are free to spend time in the location where they had experiences or sessions with the drug versus another location that never was associated with a drug. The amount of time spent is directly compared, or a transformed metric can also be used. A greater time spent in a drug-paired context indicates an association that was formed, and implies that subjects "prefer" to be in a context that signals a euphoric drug or pleasurable experience. Non-contingent administration is thought to better model the doses that drug addicts actually take, along with their neurophysiological changes, given that real-world drug infusions are usually large "hits" rather than many small ones that are modeled by operant

paradigms (Fivel, 2011; Kmiotek et al., 2012). The differences between administration protocols may also result in different experimental observations, as the dosages and contexts are different.

First, it's true that different contexts while same dosage of narcotics can have dire consequences for users and test subjects, showing that drug tolerance can even undergo classical conditioning (Vila, 1989; Gerevich et al., 2005). Contingent administration models yield greater plasma corticosterone levels, suggesting an altered endocrine response to self-dosages (Galici et al., 2000). It's possible that intra-striatal dopamine levels in the accumbens shell may be raised compared to the core for non-contingent receipt of drugs of abuse, however, there exists evidence to the contrary purely looking at dopamine the accumbens shell (Lecca et al., 2007; Howard et al., 2008). Interestingly, there is evidence that non-contingent pretreatment doses of cocaine can inhibit or invigorate operant responses for drugs, dependent on the size of the non-contingent dosage (Markou et al., 1999).

The aforementioned neurophysiological processes may govern a descent into addiction, or a set of compensatory mechanisms within linked brain regions. However, current knowledge about what multiple brain regions and interacting memory systems they govern together during the transition to addiction is limited due to the nature of single unit or smaller-scale multisite electrode recording technology, and typically only spans a small area within a brain region (Buzsaki, 2004; Paz et al., 2009; Stevenson and Kording, 2011). Electrophysiological recordings in motor related regions such as the cortex and striatum may yield operant or task performance-related artifacts that have little to do with the encoding of a drug memory. Given this, the task we designed to provide cocaine to awake-behaving mice accommodates our novel, high-density multiregion electrodes while they are head-fixed on a uniaxial treadmill, learning a non-contingent protocol. Head-fixing ensures the probes stay intact and in the same position when placed on a mounted micromanipulator; in this case we chose to initially attempt to make hippocampal recordings while doing a CPP protocol. We hypothesized that drug-dependent

changes in firing patterns of hippocampal place cells, neurons that are active when the subject enters a particular place, would occur (Lansink et al., 2012; Buzsaki and Moser, 2013). A key distinction between tetrode recording in the hippocampus or usage of commercially available silicone probes and our technology is that our greater sampling power may yield insights on relative changes in ensemble activity across multiple subregions in the hippocampus, as well as potential downstream targets in the striatum.

To even perform a CPP task in a head-fixed mouse model was a first step to this goal, as so far no research has been published outlining this procedure, although some group have used freely moving rats in conjunction with tetrode recordings in VR, albeit not while studying the effects of drugs of abuse (Aghajan et al., 2015). If the behavior task was successful, we would then proceed to make recordings. Since the treadmill we have previously built is uniaxial and mice are head-fixed, the conditioning and test portions of the task cannot rely on real-world environment preferences (Shobe et al., 2015; Bakhurin et al., 2016). In the current study, we constructed a novel VR environment using scent, vision, and locomotion for assessing CPP with cocaine as a drug reinforcer. We observed that although half the mice tested robustly exhibit place preference, others differed markedly in locomotor response, rendering usage of VR CPP as a readout of non-contingent administration less than ideal. Further options to enable head-fixing and assessing behaviors coupled to neurophysiology of the transition to drug addiction are discussed.

Materials and Methods

Virtual Reality Conditioned Place Preference Pretest/Test Chamber

Previously, other labs have demonstrated VR experiments using a 3D modeling program, Platinum Arts SandBox, to generate an environment mice can navigate via computer peripheral control while on a treadmill. In this case, an optical mouse detects the motion of the 200 mm

diameter treadmill/ball that the mouse walks on, and provides information to Labview. We've modified this procedure to use three monitors in front of our uniaxial treadmill for space and simplicity, instead of a toroidal screen (Fig. 1A) (Harvey et al., 2009). Typical VR setups use biaxial motion on a floating treadmill supported by pressurized air, which been shown to represent a twofold problem in piloted data (not shown) in our lab. Such floating ball setups produce audible noise which may confound behavioral results, as well as increase difficulty in detecting subject position in the virtual environment via requiring a server and client computer along with technical networking protocols. To more simply detect uniaxial motion, we created a single recursive hallway in Platinum Arts to serve as a CPP pretest/test chamber. This hallway was separated into four visually distinct quadrants mapping to 250 mm each when accounting for subject avatar motion (Fig. 1B). Autohotkey was activated with a custom script on the VR computer to ensure uniaxial movement on the real ball would correspond with the virtual environment. Inside the recursive hallway were hidden sound files that increased in frequency with increasing distance from the starting point (Fig. 1B). Sound output of the VR program was fed directly into a LabView mini DAQ, which performed a Fourier transform on the audio data in LabView. As sound files changed due to navigation through the virtual hall, changing position was therefore tracked and also converted into velocity. During the pretest and CPP test, as subjects moved through the virtual hallway, quadrant-based odors were emitted from a custom olfactometer (Fig 1A&B) (Shobe et al., 2015). Only three odors were used: Citral and isoamyl (banana) odors would be paired with conditioning injections, and emit only in quadrants 2 and 4, respectively, while eugenol (clove) was emitted in unpaired or neutral quadrants 1 and 3 to simulate a center chamber (Tsai et al., 2009). Since quadrants 1/3, 2, and 4 were visually distinct already, adding odors that only emitted while subjects were in them further enhanced this distinction, as with multiple different sensory experiences in real CPP environments (Tzschentke, 2007). At the beginning of each pretest, conditioning day, or test in the virtual hall,

the subject was prevented from movement via Autohotkey control of the avatar and kept in a black virtual environment until the lock was released by the investigator. During conditioning, a recursive hall representing either quadrant 2 or quadrant 4 was used instead of the 4-quadrant hall. Only the odor associated with that quadrant would be emitted during conditioning.

Subjects

All procedures were approved by UCLA's animal care committee's regulations. We used singly housed male C57Bl/6J mice; n = 6 receiving alternating cocaine and saline injections, The Jackson Labs, 12–16 weeks old. Animals underwent surgery under isoflurane anesthesia in a stereotactic apparatus to implant stainless steel head restraint bars bilaterally onto their skulls using dental cement (Shobe et al., 2015). This set of animals was used only to pilot the behavior set up, and no further survival surgeries were performed on them

Pretest and Cocaine conditioning

One week after the first surgery, animals began habituation to head restraint and the behavioral conditioning/testing room for four days, with one 30 minute session per day. Animals were mounted with the head bar bracket on top of a 200 mm diameter, uniaxial, Styrofoam treadmill /ball that was free to rotate forwards and backwards. No other stimuli were presented during habituation. On the day following habituation, fully awake head-restrained animals were head-fixed and mounted on the treadmill with the 4-quadrant VR environment as previously described for a 30 minute pretest. Time spent in each quadrant was monitored and used in the calculation of a quadrant preference index. To ensure consistency across training and behavioral testing we also inserted the needle for conditioning during the pretest session, but it was disconnected from any fluid delivery system. The day following the pretest, fully awake head-restrained animals were conditioned over 6 days to injections of cocaine or saline, paired

with either the citral or isoamyl odor in their appropriate recursive quadrant as previously mentioned. Each daily conditioning session for the drug group was devoted to either cocaine or saline, but never both on the same day. The drug group received alternating cocaine (20 mg/kg, 100 µl, s.c.) or saline (100 µl, s.c.) infusions administered over a 1 min period, 5 minutes after entering the environment. The infusions were given by means of a syringe pump connected to a 27.5 gauge syringe needle inserted subcutaneously. We removed the needle after each session. Conditioning started with saline on day 1 and ended with cocaine on day 6. All cocaine or saline administration sessions were paired with the animal being in the appropriate recursive quadrant and a continuously pulsed (150ms on, 75% duty cycle, 5 hz) presentation of odorized air, starting 5 min pre-injection and ending 25 min post-injection, at which point the animal was removed from the conditioning room and returned to its home cage in a vivarium housing facility. Olfactory cues were introduced via an olfactometer that received subject position and quadrant data from LabView. This olfactometer bubbled air (0.15 l/min) through aromatic liquids (citral, isoamyl acetate, or eugenol) diluted 1:10 in mineral oil (Sigma-Aldrich), and mixing this product with a 1.5 l/min stream of air. This mixture flowed through a tube passing 15 mm away from, and perpendicularly to the animal's nose, with a 5 mm opening in the tube aimed at the nose. Half of the animals were counterbalanced and received the opposite drug-odor type pairing. On the day following the final cocaine conditioning, animals abstinent from their last injection of cocaine for 24±3 hrs underwent the CPP test in the pretest/test VR environment. As on the pretest day, they were head-fixed and mounted on the treadmill with the 4-quadrant VR environment for a 30 minute CPP test. Time spent in each quadrant was monitored and used in the calculation of a preference index. To ensure consistency across training and behavioral testing we also inserted the needle for conditioning during the test session, as before.

Behavioral data analysis and statistics

To quantify behavioral responses during cocaine or saline conditioning, we calculated the mean post-injection treadmill ball rotation per animal and subjected mean per-day results to a two-way ANOVA. To quantify CPP during abstinence, for each animal on the pretest and test we calculated the change in speed and position to obtain a fractional amount of time spent in each quadrant. These data were subject to a one-way ANOVA (Friedman test) corrected for multiple comparisons (Dunn's test). We then calculated a preference index for the pretest as follows:

$$\text{Pretest preference index}_{ab} = (\text{time in quadrant}_a / (\text{time in quadrant}_a + \text{time in quadrant}_b))$$

These calculations were repeated for all combinations of quadrants and subject to the same Friedman test as before. Statistics were conducted in GraphPad Prism 6, unless otherwise noted.

Results

Psychomotor sensitization observed through cocaine conditioning in a VR environment

Upon conditioning head-fixed mice in a VR environment (Fig. 1A&B) for six days (Fig. 1C), we observed a classic trend towards psychomotor sensitization among the six treated mice. This is observed as an increased total distance traveled (rotational movement) on the ball after the initiation of each conditioning injection on cocaine injection days compared to saline injection days (Fig. 2A, two-way ANOVA, effect of treatment type: $F_{(1,10)} = 7.17$, $p = .023$; effect of treatment day: $F_{(2,20)} = 1.96$, n.s.; interaction effect: $F_{(2,20)} = 0.275$, n.s.).

After conditioning there is a large variability in how mice exhibit CPP in VR

Among all animals on the pretest day, prior to conditioning, we found no significant difference in the fraction of time spent in any particular quadrant (Fig. 2B, Friedman test, p = 0.874, Dunn's correction for multiple comparisons, all n.s.). Additionally, tested mice didn't have any statistically significant group preference for any quadrant over any other quadrant (Fig. 2C, Friedman test, p = 0.993, Dunn's correction for multiple comparisons, all n.s.).

Among all animals on the test day, there was an observable and statistically significant difference among all groups in the fraction of time spent in quadrants, even while accounting for counterbalanced animals; however, we couldn't obtain statistical significance while performing multiple comparisons (Fig. 2D, Friedman test, p = 0.035, Dunn's correction for multiple comparisons, all n.s.). We also found a statistically significant difference among all groups in their preference indices, but similarly most groups failed the multiple comparisons test (Fig. 2E, Friedman test, p = 0.003, Dunn's correction for multiple comparisons, all n.s. except for the preference index $_{3-1}$ to preference index $_{4-3}$ comparison).

While testing animals, we noticed that 3 of them had a tendency to remain running through the VR chamber even when encountering the cocaine paired quadrant during the test (data not shown), a behavior documented in CPP studies (Michel and Tirelli, 2002). This resulted in a very wide distribution of fraction of time spent in that chamber after testing (Fig. 2D), making the result of multiple comparison testing less significant, as well as interfering with the preference index results, as only 1 out o 15 multiple comparisons were significant, when we expected all comparisons with the cocaine quadrant (quadrant 4) to be significant (Fig. 2E). These results indicate that classic psychomotor sensitization with cocaine can be achieved in VR environments and reward-related stimuli can engage pathways that govern CPP in VR, but we cannot say with absolute certainty that the behavior we are eliciting is exactly identical to traditional CPP nor is it reliably replicable in our sample size.

Discussion

The current study aimed to establish a head-fixed VR CPP paradigm to use in conjunction with high-density electrophysiology. With CPP as a behavioral readout of an aspect of reward circuitry, non-striatal elements of the basal ganglia loop such as the hippocampus or ventral hippocampus would be amenable to recording alongside the striatum in order to gauge how glutamatergic regions and striatal regions simultaneously encode drug contexts.

In order to assess how similar our VR environment is in terms of conditioning mice to real-world conditioning environments, we established that mice undergo classic psychomotor sensitization using our virtual odor and visual stimulus quadrants. We note that movement overall under both treatment types increased and that we did not reach statistical significance between cocaine and saline days for each pair of days (Fig. 2A), and this may be an issue with sample size, insufficient number of conditioning days, placebo effect on saline days, or an issue in how the task translates into VR by paring down the possible conditioning stimuli to just the room itself, odor, and visual stimuli. However, we remain confident that psychostimulants can work in our conditioning setup, especially in head-fixed mice, given that the treatment itself resulted in an expected and statistically significant change in locomotion during conditioning. Improvements in conditioning could be made by enhancing the dosage, and increasing the numbers of conditioning days to ensure that the associations are strengthened.

Our testing setup appears to be neutral to head-fixed mice prior to conditioning, given that there are no statistically significant differences in time spent in any quadrant, nor any associated weight in preference indices during the pretest (Fig. 2B&C). Despite both measures of preference changing significantly upon the test day (Fig. 2D&E), we cannot conclude definitively that what we observe is classical CPP, given that all our tests also failed multiple comparisons, save for one. Additionally, half of our animals showed no overt sign of preference and exhibited greater locomotion by running through each quadrant, rather than remaining still

in the cocaine-paired quadrant (Fig. 2D), suggesting a placebo effect or perhaps a sign/goal tracking divergence in these mice (Flagel et al., 2009; Singer et al., 2016). It has been established that in mice, within a limited subset of subsets of measured behaviors, diverging behavioral responses to predictive cues exist, in about half of mice (Flagel et al., 2011).

Since it has been shown that odors without visual stimuli can result in conditioning when paired with an unconditioned stimulus (US) (Komiyama et al., 2010; Shobe et al., 2015), the next step for these experiments is to remove potential confounds that come with VR environments, as visual stimuli and the head-fixed nature of the experiment may be unsettling to mice, making the task more difficult to perform and thus introducing unwanted and uncontrollable variability into the behavior. Odor presentations that are associated with cocaine paired cues be what drive the differing locomotor responses we have seen, and such conditioned locomotion could be a better representation of a conditioned response while head-fixed, similar to conditioned approach or autoshaping. The next electrophysiological targets would be regions that focus on movement planning, in that case, rather than position in an environment. Overall, the continued usage of head-fixed murine models in addiction studies appears promising. High-density electrophysiology of the reward pathway in this context has heretofore not been done, and the demonstration that mice can exhibit important aspects of Pavlovian learning in addiction models such as CPP or conditioned approach is an important first step.

Figures

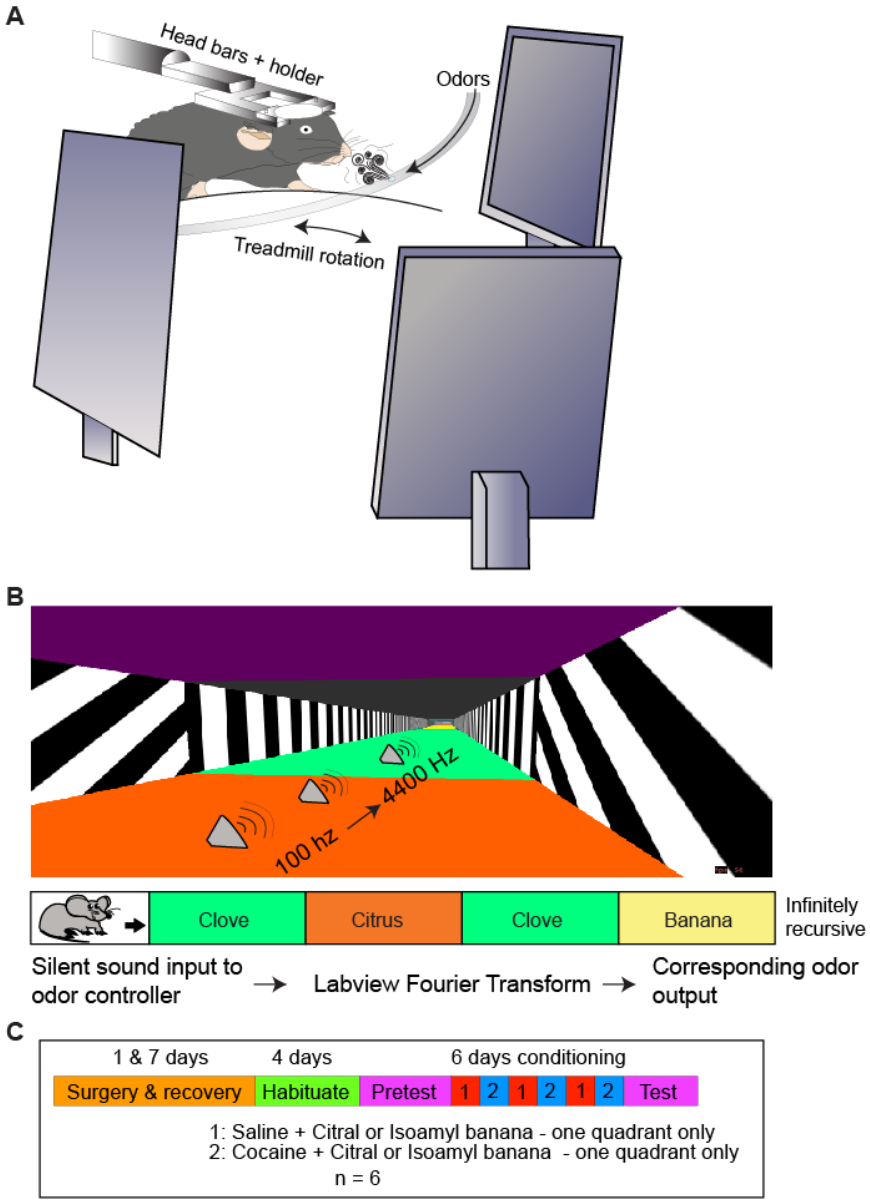


Figure 1. CPP in VR experimental setup and design. **A,** Awake, head-fixed mice receive visual input from monitors around them, while odors simultaneously help to signal contexts. Behavior was monitored as ball rotational velocity. **B,** As mice move through the virtual hallway in front of them, they encounter four quadrants containing different visual patterns and odors. Sound files play tones of increasing frequency with increasing avatar distance in the hall, and the voltage from the audio out of the VR machine is linked directly to a Fourier Transform script that detects changing audio and thus position. This controls odors and changes them based on where mice are in the hall. **C,** The daily experimental paradigm to induce CPP involves headbar surgery, recovery, and habituation to the ball and behavior room. Following that, a pretest with exposure to all the quadrants in VR establishes baseline quadrant preferences. Mice are then conditioned for 6 days on 20 mg/kg cocaine and then when abstinent for 24 hr from cocaine, allowed to explore the VR environment and the quadrants with odors again to assess CPP.

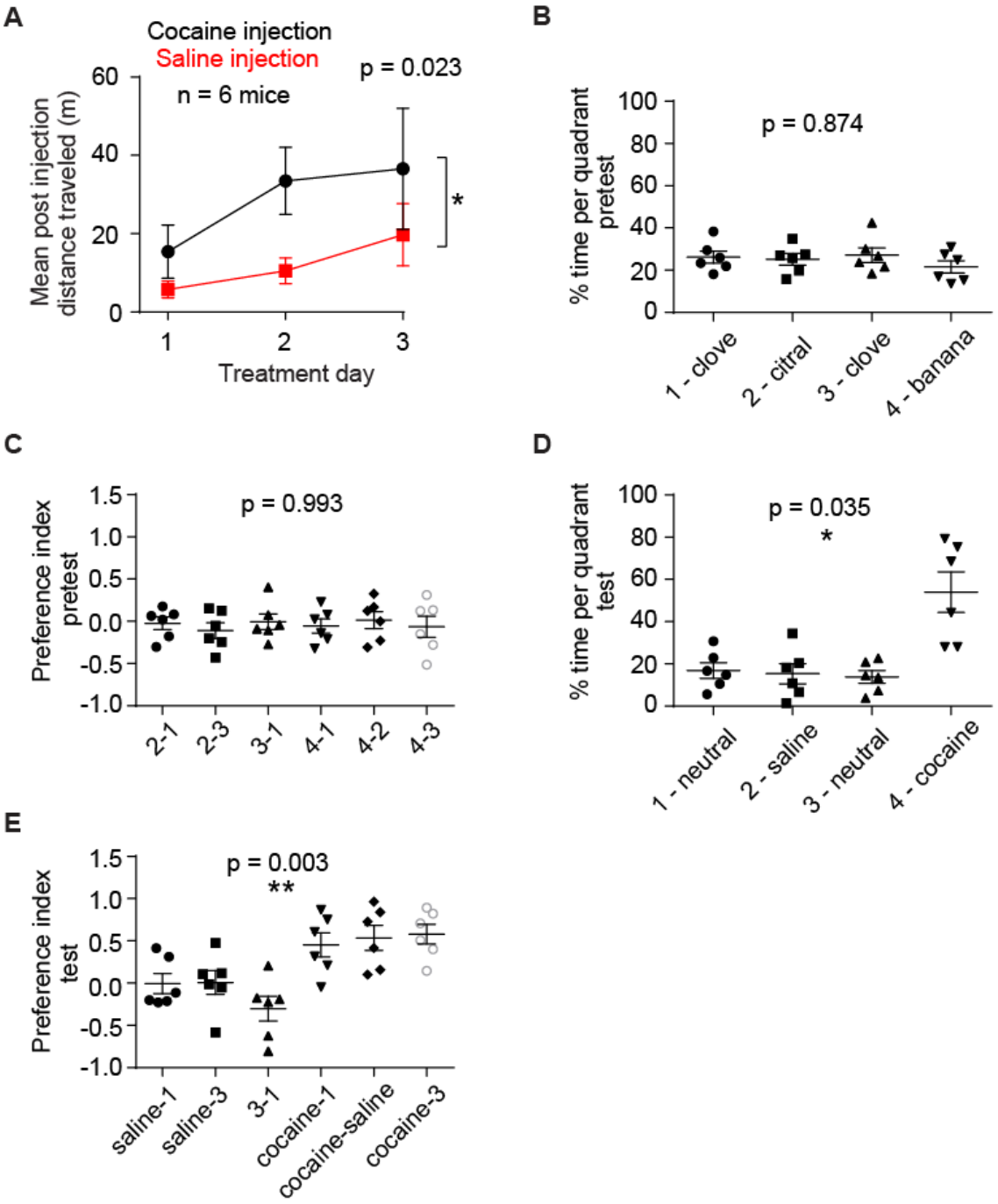


Figure 2. Demonstration of psychomotor sensitization and aspects of CPP in head-fixed VR. **A,** Post-injection treadmill distance traveled showed a significant effect of treatment type (two-way ANOVA, $F_{(1,10)} = 7.17$, $p = .023$). **B,** During the pretest , mice didn't stay in any particular quadrant longer than any other (Friedman test, $p = 0.874$) and in **C,** did not exhibit a preference of one quadrant over any other quadrant (Friedman test, $p = 0.993$). **D,** There was a difference among groups in the test day for time spent in quadrants, but no definitive preference for the drug-paired quadrant (Friedman test, $p = 0.035$, Dunn's correction for multiple comparisons, all n.s.). **E,** mice appear to have a difference in preference to the cocaine quadrant, but again fail at most multiple comparison tests to clearly show preference (Friedman test, $p = 0.003$, Dunn's correction for multiple comparisons, all n.s. except for the preference index 3-1 to preference index 4-3 comparison).

CHAPTER 2

COCAINE CONDITIONING RESULTS IN VARIABLE PAVLOVIAN APPROACH BEHAVIORS IN HEAD-FIXED MICE

Abstract

Cocaine, being a highly rewarding and euphoria-inducing drug of abuse, has many neurophysiological and behavioral effects. Interacting elements of dopaminergic and glutamatergic dysregulation may be at play during the transition to addiction, but multiregion interactions have been difficult to characterize or link to behavior. Having demonstrated that head-fixed or head-restrained mouse protocols are compatible with substance abuse research, particularly with non-contingent cocaine administration, we continue investigating the underlying interacting neural systems that are associated with addiction-related behaviors. In order to do so, we improve upon our previous behavior paradigm and simultaneously paired them with high-density electrophysiology of the frontal cortex and striatum. Similar to previous behavioral results, we observe that Pavlovian approach behaviors are widely variable in mice without following a normal distribution, making them difficult to elicit via cues in an un-rewarded experimental context with head-restrained mice. Additionally, our electrophysiology data show largely movement-related firing patterns, rather than only cue-elicited firing patterns, making them noisy in the context of trying to uncover drug-memory related firing. These results suggest that further refinement is necessary in head-restrained behavioral approaches in non-operant paradigms.

Keywords: cocaine, head-fixed behavior, basal ganglia, Pavlovian approach, electrophysiology

Introduction

Previously we have shown that CPP-like behaviors can be shown in head-restrained mice that have undergone associative learning with cocaine as the conditioned reinforcer (cocaine conditioning). There were two primary problems with the previous results, however. The first issue was that in both tests, we obtained poor multiple comparison results despite accommodating for our low number of subjects, and the underlying cause of that was a lack of convincing replicability in locomotion and quiescence. In order to move forward with the usage of high-density electrodes in a drug-mediated Pavlovian task, we decided to improve our task in the habituation, conditioning, and probe stages to both simplify what was required of mice, and ideally make the resultant behaviors more robust and replicable.

The probe task we changed to relies on observations of Pavlovian approach (Pavlovian conditioned approach) or autoshaped/sign-tracking responses, which differ from manifesting CPP, but are conditioned similarly to CPP in our task (Brown and Jenkins, 1968; Newlin, 1992). An autoshaped behavior involves the expression of sign-tracking, where animals attribute incentive salience, that is “desire/want”, to a CS, turning a CS into a rewarding stimulus on its own, rather than a mere predictor of a US (Fitzpatrick et al., 2013). Autoshaping explicitly means that an approach behavior, or in the classical case of pigeons, pecking at a CS, is arrived at incrementally by repetitions and reinforcement of such innate responses. In mice, approach and contact in order to initiate consummatory behaviors with a CS would fall under the classification of autoshaping, but in most experimental cases, the CS is a temporally discrete cue.

Additionally, the very nature Pavlovian approach implies that there’s some operant factor in the behavior, even if the subject isn’t necessarily rewarded for approaching or performing an innate consummatory behavior at or near an approached reward-associated cue (Williams and Williams, 1969; Wasserman et al., 1974). This makes a clear overarching distinction between

purely Pavlovian and purely operant tasks difficult to make, as for example, it has been observed that CPP is “an awkward form of autoshaping” as some form of external CS (albeit response-independent) eventually yields a motor response towards the CS (Newlin, 1992). Although the literature may describe some similarity between the varieties of conditioned approach behaviors named, here we explicitly define our form of Pavlovian approach, given that there is a distinct caveat with the paradigm we’ve created. Unlike most protocols using a temporally discrete CS, we still condition with a contextual cue similar to CPP (here, only a constant odor), but in the probe we have removed the contextual visual and olfactory environments that depend on subject changing spatial positioning, and made the probe/test consist of randomly presented, discrete olfactory cues that recalls the contextual conditioning cues or are novel/new cues. As described previously in our experiments, subjects experience a contextual cue during cocaine treatment that is not temporally discrete, but represents a unique context that distinguishes the treatment from the saline context (Curzon et al., 2009). During CPP probe trials after conditioning, presenting a choice of contexts to the subject allows the experimenter to observe if the preference has manifested, but successful behaviors require that mice both move and stop appropriately. For our new test protocol, discrete drug-associated, unpaired, or saline-associated cue presentations in front of the animal may allow observation of a more simple and natural approach response that may undergo autoshaping with cocaine conditioning, similar to classic behavior experiments in rats using intracranial stimulation both inside and outside the basal ganglia as a US (Peterson et al., 1972). This would also work without the complications that come with CPP and included visual stimuli in a VR environment that may unsettle the subject and confound behavior, while also allowing for a comparison to novel cues that don’t share any association.

We’ve modified the behavioral habituation procedure to assist mice while running on the ball/treadmill on their first session. Rather than let mice get accustomed to the ball reacting to

uncoordinated movements, for the first five to ten minutes of habituation, we rotated the ball forwards for them, so they can immediately familiarize themselves with how the rotation of the ball feels and more readily manifest psychomotor sensitization and potential conditioned approach behavior. We also extended the conditioning with a slightly lower dose of SQ cocaine, going from three days to four days alternating saline injections with 15 mg / kg of cocaine instead of 20 mg / kg, in order to improve learning through repeated trials with experiencing a potent reinforcer and better manifest Pavlovian approach (Prus et al., 2009). The conditioning itself had no visual stimuli, but still retained the constant puffs of odorized air in front of the subjects, with each odor paired with a corresponding drug or vehicle treatment (citral or isoamyl banana). Finally, rather than use a VR environment in our test, trials consisted of bursts of odorized air (using the previous odors) that were randomly presented to mice with a variable ITI, in order to test for conditioned approach behaviors to odors only. Interspersed among conditioning-associated odor trials on the test days, we also provided novel clove odor trials to verify that mice have an elevated behavioral response to a drug-paired odor specifically, and not any odor that may elicit a novelty response (Crusio et al., 1989). We also removed the pretest day from our training in order to avoid tampering with the expression of the proper behavior due to latent inhibition, a known phenomenon whereby previously experienced stimuli take longer to become conditioned stimuli (Lubow and Moore, 1959) . We consider this a sound decision, given that in our previous experiments we've seen no evidence for an innate preference of odors in mice, either in terms of stimulating locomotion or in place preference.

The last change made to the protocol was the addition of a conditioning group that incubated cocaine craving for 21 days after their final conditioning day. These mice were not handled or brought back into the laboratory for 21 days so as to not invoke any memories of treatment or handling, however, they underwent tests the same as other mice. It's been observed that while abstinent from drugs after intense usage, both PFC and amgydala efferents

to the nucleus accumbens generate calcium permeable AMPA receptors within the latter, and BDNF (brain-derived neurotrophic factor) increase throughout the brain, particularly the VTA and accumbens (Lee et al., 2013; Ma et al., 2014; Geoffroy and Noble, 2017). We reasoned that these animals should have a heightened response to drug cues, particularly because of how aberrantly increased TrkB (BDNF) signaling is observed in animals undergoing withdrawal, which, like having extra cation permeable channels in the accumbens, would also increase cue-responsive excitability locally (Kafitz et al., 1999; Ren et al., 2015).

Concurrent with probe trials in our odors-only protocol, we performed 512 channel extracellular electrophysiology of the cortex and striatum of 24 hr post-conditioning mice as well as mice incubating craving using a custom, nanofabricated, silicon electrode array. Due to technological limitations at the time of experimentation, we were not able to make probes with closely spaced shafts, so the high number of electrodes was spread across the right mPFC and into the orbital cortex. In the striatum, the shafts extended from the edge of the right accumbens into the right dorsolateral striatum.

We posited that there would be population level changes in mean firing rate in both regions in response to cocaine and saline paired cues, but not novel cues, and changes in firing would be more dramatic in mice incubating craving. Since these techniques had yet to be applied in an addiction model, we deemed that such a simpler metric may be more appropriate in this first-pass analysis, and we discuss caveats of this approach as well.

We found that our mice conditioned similarly to the previous experiments, as we had expected. However, despite making our probe task simpler, we observed a bimodal distribution in behavioral responses on the test day, in that we saw mice that in response to cues ran quickly and those that were mostly quiescent. Mice that ran, both in the incubating craving group and in the 24 hr-post conditioning group, showed sustained elevated population firing in response to the drug paired cue amongst all projection neuron and interneuron types that we

could discern in the cortex and striatum. However, we were unable to untangle these data from simple running-related responses, an important caveat. These uncertainties made results difficult to interpret, as another factor other than the cue is largely controlling the expression of the behavior, and the firing rates observed may be altered by the expression of the behavior or slight twitches, rather than represent an internal state or memory code. We discuss potential remedies to fix both the unclear elements of behavior and electrophysiology in these experiments, as well as issues in design of the probe to be fixed.

Materials and Methods

Subjects

All procedures were approved by UCLA's animal care committee's regulations. We used singly housed male C57Bl/6J mice; n =12 receiving injections, The Jackson Labs, 12–16 weeks old. Animals underwent surgery under isoflurane anesthesia in a stereotactic apparatus to implant stainless steel head restraint bars bilaterally onto their skulls using dental cement (Shobe et al., 2015). N = 6 of these mice were to be conditioned normally with electrophysiology to follow 24 hr after their last dosage ("24 hr group"), while n = 4 mice were left in their home cages for 21 days after their last dosage ("incubation group"). N = 2 mice were used as controls that only ever received saline injections ("control group"). Finally, Animals were anesthetized with isoflurane for a second surgery on the evening prior to the day of the recording session to make a craniotomy over the right hemisphere's anterior striatum and PFC. The dura mater was opened to facilitate insertion of microprobes during the recording session. An additional craniotomy was made over the posterior cerebellum for placement of a silver/silver chloride electrical reference wire. The craniotomies were covered with silicone elastomer sealant (Kwik-Cast, WPI) to seal off the exposed skull.

Habituation and Cocaine conditioning

One week after the first surgery, animals began habituation to head restraint and the behavioral conditioning/testing room for four days, with one 30 minute session per day. All animals were mounted with the head bar bracket on top of a 200 mm diameter, uniaxial, Styrofoam treadmill /ball that was free to rotate forwards and backwards. For the first five to ten minutes of habituation, forwards motion was assisted on the ball. No other stimuli were presented during habituation. The day following habituation, fully awake head-restrained animals were conditioned over 8 days to injections of cocaine or saline, paired with either the citral or isoamyl odor constantly being puffed in front of them. Each daily conditioning session for the drug group was devoted to either cocaine or saline, but never both on the same day. The drug-treated group received alternating cocaine (15 mg/kg, 100 µl, s.c.) or saline (100 µl, s.c.) infusions administered over a 1 min period, 5 minutes after being in the behavior room. The infusions were given by means of a syringe pump connected to a 27.5 gauge syringe needle inserted subcutaneously. We removed the needle after each session. Conditioning started with saline on day 1 and ended with cocaine on day 8. All cocaine or saline administration sessions were paired with experiencing the appropriate continuously pulsed (200ms on, 75% duty cycle, 4 hz) presentation of odorized air, starting 5 min pre-injection and ending 25 min post-injection, at which point the animal was removed from the conditioning room and returned to its home cage in a vivarium housing facility. Olfactory cues were introduced via an olfactometer that bubbled air (0.15 l/min) through aromatic liquids (citral, isoamyl acetate, or eugenol) diluted 1:10 in mineral oil (Sigma-Aldrich), and mixing this product with a 1.5 l/min stream of air, but with eugenol reserved for tests as a novel cue. This mixture flowed through a tube passing 15 mm away from, and perpendicularly to the animal's nose, with a 5 mm opening in the tube aimed at

the nose. We monitored ball velocity via the same optical mouse setup as previously, and converted this to distance traveled to assess psychomotor sensitization.

Behavioral test

The craniotomy surgery was carried out 7-8 hrs after the final conditioning session, and mice were allowed to recover at least 16 hours. Behavioral testing and electrophysiological recordings were carried out simultaneously in drug-free conditions, in animals abstinent from their last injection of cocaine (24 hr group) or saline (control group) for 24±3 hrs. Mice were head-fixed, and to ensure consistency across training and behavioral testing we also inserted the needle for conditioning during the test session, but it was disconnected from any fluid delivery system. Mice stayed on the ball for 10 minutes of quiescence once the electrode array was in place to capture spontaneous activity and motion. They then experienced randomized 5 s puffs of odorized air using three distinct odors: citral, isoamyl acetate (banana), or eugenol (clove), separated by a ~30 s ITI. Each odor was controlled so that more than three presentations of the same odor in a row would be prevented, and odors were presented a minimum of 30 times. We obtained rotational ball velocity in response to these cues. Mice in the incubating group were allowed to remain in their home cages for 21 days, and were then subject to the same testing protocol.

Electrophysiological recordings

A silicon microprobe (Shobe et al., 2015) was assembled targeting the frontal cortex from mPFC to orbital cortex (see Fig. 2A) and striatum from (256 electrodes per area, total of 512 electrodes). Each area was targeted with 5 silicon prongs spaced ~0.35 mm apart, each containing ~51 electrodes that were arranged in a hexagonal array pattern spanning 1.25 mm along the dorso-ventral axis. Electrodes had an area of 100 μm2 and were gold-plated (non-

cyanide gold solution, Sifco) to an impedance of 0.1-0.5 MΩ. 1-2 hrs prior to recording, the silicon microprobes were coated with a drop of fluorescent dye (DiD, Invitrogen) to assist with histological confirmation of their position. Animals were then mounted on the head restraint system as described previously. An electrical reference wire was placed on the cerebellar surface, covered in ACSF-saturated water absorbing foam (Gelfoam) to improve electrical contact, and sealed with silicone elastomer (Kwik-Cast, WPI). The silicon microprobes were slowly inserted to stereotactically defined coordinates with a motorized micromanipulator (MP-285, Sutter Instruments). The tip position of the most medial shaft in the mPFC was targeted to 2.20 mm anterior, 0.250 mm lateral, and 3.60 mm ventral relative to bregma in the right hemisphere. The tip of the most medial shaft in the VS was placed in the same hemisphere at 1.10 mm anterior, 0.80 mm lateral, and 4.25 mm ventral relative to bregma. For a visualization of probe insertions, see Fig. 2A. The insertion was monitored with a surgical microscope. After reaching the target depth a drop of mineral oil was placed on the exposed cortical surface, and a 45 minute settling period elapsed before beginning the electrophysiological data acquisition and behavioral testing (see the previous section). We monitored electrophysiological data at a sampling rate of 25 kHz per electrode, together with treadmill ball rotation and olfactory cue delivery time at a sampling rate of 10 kHz. Following each recording, the microprobe was cleaned in trypsin solution (Invitrogen), rinsed with deionized water and ethanol, and reused throughout these experiments. Note that for the recording of the final 24 hr group animal, the electrodes were no longer intact, leaving 5 recordings for that group, rather than 6; behavioral data were still saved, however, allowing us to still use those data for analyses.

Histology

Coronal brain sections were sliced at 100 μm on a vibratome and individual sections were placed in order into a 24-well plate containing ice-cold PBS solution. We then performed

immunohistochemistry using standard procedures on the mPFC and striatal sections to determine the placement of the silicon prongs in each region. Sections were stained for neuronal nuclei with chicken-α NeuN primary antibodies (D3S3I, Cell Signaling Technology) and fluorescent α-chicken secondary antibodies (Alexa 488, Jackson Immuno Research). DiD, which diffusively labeled tissue near the probe insertion sites, was used to estimate the final probe position. Each of the 512 recording sites was assigned a coordinate in 3D Cartesian space based on the expected location relative to bregma.

Unit classification

Spike sorting was performed using a spike waveform template matching algorithm (Shobe et al., 2015). Each unit was assigned an estimated 3D coordinate as well as a histologically determined brain region. The estimated position coincided with the recording site exhibiting the highest spike amplitude for that unit. Finally, units were classified as principal neurons or interneurons in their respective brain regions. Striatal units with a minimum baseline rate of 0.02 Hz were classified as putative medium spiny projection neurons (MSNs), fast spiking interneurons (FSIs), or tonically active neurons (TANs), based on spike waveform peak-to-trough width, and coefficient of variation of the baseline firing rate (Bartho et al., 2004; Bakhurin et al., 2016). FSIs were characterized by a narrow spike waveform (maximum width = 0.475 ms). MSNs and TANs both have wider waveforms (minimum width = 0.55 ms, maximum width = 1.25 ms). TANs were separated from MSNs by the regularity of their baseline firing (maximum coefficient of variation = 1.5). Units in the mPFC were classified as putative FSIs or pyramidal neurons using the same spike width separation criterion described above (Tseng et al., 2006). For a visual guide of what units were typically not considered as interneurons or projection neurons, see the dotted lines on the histograms in Fig, 2B,

Behavioral data analysis

To quantify behavioral responses during cocaine or saline conditioning, we calculated the mean post-injection treadmill ball rotation velocity and used that to obtain total locomotion. To quantify the cue-evoked behavioral responses on the test day, for each animal we calculated the change in speed during the 5 s cue presentation time relative to a 1 s baseline period prior to cue onset. Unless otherwise noted, responses were averaged across all trials presented (cocaine-paired, saline-paired, or novel).

Electrophysiological data analysis

Single-unit firing rate was calculated in time steps of 50 ms, and smoothed by convolving with a Gaussian kernel (s.d. = 250 ms). Spontaneous firing activity was obtained by averaging the rate from the first 10 min of the recording, when animals received no olfactory cues and did not move on the treadmill ball. Cue-evoked activity was obtained by averaging across all cocaine-cue, saline-cue, or novel trials. To determine whether units were excited or inhibited by cues, we performed a permutation test to identify time bins whose firing rate was significantly different from a 1 s baseline period preceding the cue onset (criterion for significance: $p < 0.01$). If two or more consecutive time bins during the cue presentation period (t = 0 - 15 s from cue onset) were significantly greater (less) than the baseline, the unit was classified as excited (inhibited).

Results

Mice exhibit psychomotor sensitization without VR visual stimuli

Upon cocaine conditioning head-fixed mice with odors only for eight days, we observed a classic psychomotor sensitization among the 10 treated mice. This was again observed as an increased total distance traveled (rotational movement) on the ball after the initiation of each

conditioning injection on cocaine injection days compared to saline injection days (Fig. 1A, two-way ANOVA with Sidak's correction for multiple comparisons, effect of treatment type: $F_{(1,18)}$ = 4.77, p = 0.042; effect of treatment day: $F_{(3,54)}$ = 4.12, p = 0.011.; interaction effect: $F_{(3,54)}$ = 2.19, p = 0.10). As expected, control mice that only ever received saline injections paired with odors did not have increased running on the ball over days of treatment, nor did they run with greater preference to any treatment day (Fig. 1B, two-way ANOVA with Sidak's correction for multiple comparisons, effect of treatment type: $F_{(1,2)}$ = 0.75, p = 0.477; effect of treatment day: $F_{(3,6)}$ = 1.12, p = 0.411.; interaction effect: $F_{(3,6)}$ = 1.12, p = 0.411).

Mouse running exhibits a bimodal distribution in a simpler Pavlovian, drug-cue related task

During testing, control animals ran very little with a post-cue velocity close to 5 mm/s, and performed similarly for each odor (Fig. 1C, Friedman test with Dunn's correction for multiple comparisons, p = 0.50). Mice in the 24 hr. drug-treated group exhibited a similar result, with a very low amount of running and no difference in running preference for any cues (Fig. 1D, Friedman test with Dunn's correction for multiple comparisons, p = 0.093). Finally, mice in the drug craving incubation group behaved similarly to the other groups, with an almost imperceptible amount of running when averaged across animals, and no statistically significant preference in running for any cue (Fig. 1E, Friedman test with Dunn's correction for multiple comparisons, p = 0.431). Notably, all of the tested groups have a similar bimodal distribution in their behavior test data, as with the previous CPP experiments.

Cocaine does not alter population-level spontaneous and cue-evoked neural activity early in abstinence nor does it during incubation of craving

Our custom 3D probe inserted in the PFC and striatum simultaneously (Fig. 2A) allowed recording of clearly delineated cell types according to our classification criteria (Fig, 2B).

Recordings yielded a total of 2334 units across 11 mice (Fig. 2C), or more than 212 units / mouse and an approximate yield of 41.4% units / # electrode, consistent with previous recordings from the lab. In control mice, 32.2% of neurons fit our criteria for MSNs, 5.0% were putative FSIs, 3.5% were putative TANs, 47.5% were putative cortical pyramidal neurons, 7.2% were putative cortical fast-spiking interneurons (CFSI or Cort. FSI), and 4.6% were unclassified. In the 24 hr group of drug-treated mice, 25.6% of neurons fit our criteria for MSNs, 6.4% were putative FSIs, 1.7% were putative TANs, 46.7% were putative cortical pyramidal neurons, 11.8% were putative CFSIs, and 7.8% were unclassified. Lastly, in the incubating group, 22.5% of neurons fit our criteria for MSNs, 4.7% were putative FSIs, 3.2% were putative TANs, 52.2% were putative cortical pyramidal neurons, 7.9% were putative CFSIs, and 9.5% were unclassified. Counts of units collected are listed in the table in Fig. 2D, with subject abbreviated at the top. Due to difficulty in discerning TANs from MSNs in the striatum from purely electrophysiological data and their low yield (the lowest among all cell types, see table in Fig. 2D), we do not discuss TAN properties or patterns in subsequent analyses.

We observed no significant differences in resting firing rates versus the control condition for any projection or interneuron type examined, contrary to what we expected, given many observations of cortical hypoactivity after cocaine administration (Fig. 3A, Kruskal-Wallis ANOVA with Dunn's correction for multiple comparisons, all four cell types compared to their respective controls, $p > 0.05$) (Trantham et al., 2002; Chen et al., 2013). When looking at triggered firing in all conditions, for each cell type we plotted normalized firing rate of each cell in all animals and ordered their rate descending by latency of response to each cue (Fig. 3B, only control data raster for MSNs shown as an example). A timeseries of mean triggered firing in MSNs is shown at the bottom left of Fig. 3B and is repeated for each condition and cell type through 3B, 3C, and 3D, with cell types labeled. From these timeseries data, we qualitatively and quantitatively saw neither statistically significant excitation nor inhibition following receipt of

cues in controls and both experimental groups. Additionally, no significant difference in change of firing was found from the cues in the drug-treated subjects vs. controls (data not shown). We also qualitatively found that receipt of odor tended to be accompanied by a slight uptick in population firing, but was not limited to the drug cue when averaged among mice.

Discussion

Although we were able to demonstrate conditioning to odors only without visual stimulation, we did elicit the autoshaped behavior in all animals, rather again we noticed that roughly half of the tested mice tended to run no matter what the cue or conditioning treatment it received was. This may result from aberrant conditioning as in sign or goal tracking, a generalized response to the behavior room, or issues with sleep/wake cycles, and time of training (Hawkins and Golledge, 2017). We reasoned that some mice don't move at all due to lack arousal during daytime hours, as they are nocturnal. Lacking an advanced setup for accommodating the natural mouse circadian rhythm, we conducted a pilot experiment with 6 mice (data not shown) by training and recording from them exclusively at night. We found the same results, a bimodal distribution of running, despite the mice being training during their maximum arousal period.

These results may suggest that even attempting to elicit a CR as simple as running may result in a behavior governed by a similar mechanism with the same downsides. Additionally, the amount of running that we could elicit is difficult to detect and may consist of no more than brief twitches, and an arbitrary distinction would need to be made to determine when bouts of true motion begin or end for further tests. We decided subsequently that the next round of experimentation to yield a behavior that was not bimodal should not need to depend on motor skills at all, and be able to be easily distinguishable from background motor behaviors like twitching, as well has promote very little background neural activity. To this end, the following

experiments utilize pupillometry, as much is known about the autonomic responses to drugs and how they are elicited even without motor activity (Rosse et al., 1995).

In the case of our electrophysiology, it appears we have consistent numbers of cells recorded across our conditions, but we cannot necessarily rule out effects from low sample size in the control condition prohibiting us from observing a significant difference in resting rates, given that mean resting rates of all neuron types were averages from two mice. However, we are confident that if there indeed was a difference between the experimental conditions and control mice, the rank-based test we used would find it. Re-sampling or bootstrapping these data is not necessarily the right choice, especially given that the SEM's of our control data are close to the experimental and outliers in neural firing are less of a concern when recording from hundreds of units. Additionally, there are only $2^N - 2$ combinations of our control data when re-sampling, and given that N is 2, the data are a poor choice for that test.

Our cue-triggered firing results are for the most part consistent with our negative behavioral result. Neither cue appears to elicit any sort of increased firing in any condition. We've omitted the novel cue from the data analysis because they were largely similar to the behavioral results and only served to establish an alternative baseline to test for generalization effects. Any increases in firing temporally close to the cue may likely be due to cortical uptick of receipt of odors or twitch motion responses in half of the mice recorded. Since recording both a motor behavior and electrophysiology in a region known to have a role in motor planning and output may create ambiguity in our results, using pupillometry in the future will limit background firing and allow for a cleaner signal to associate with a behavior.

Figures

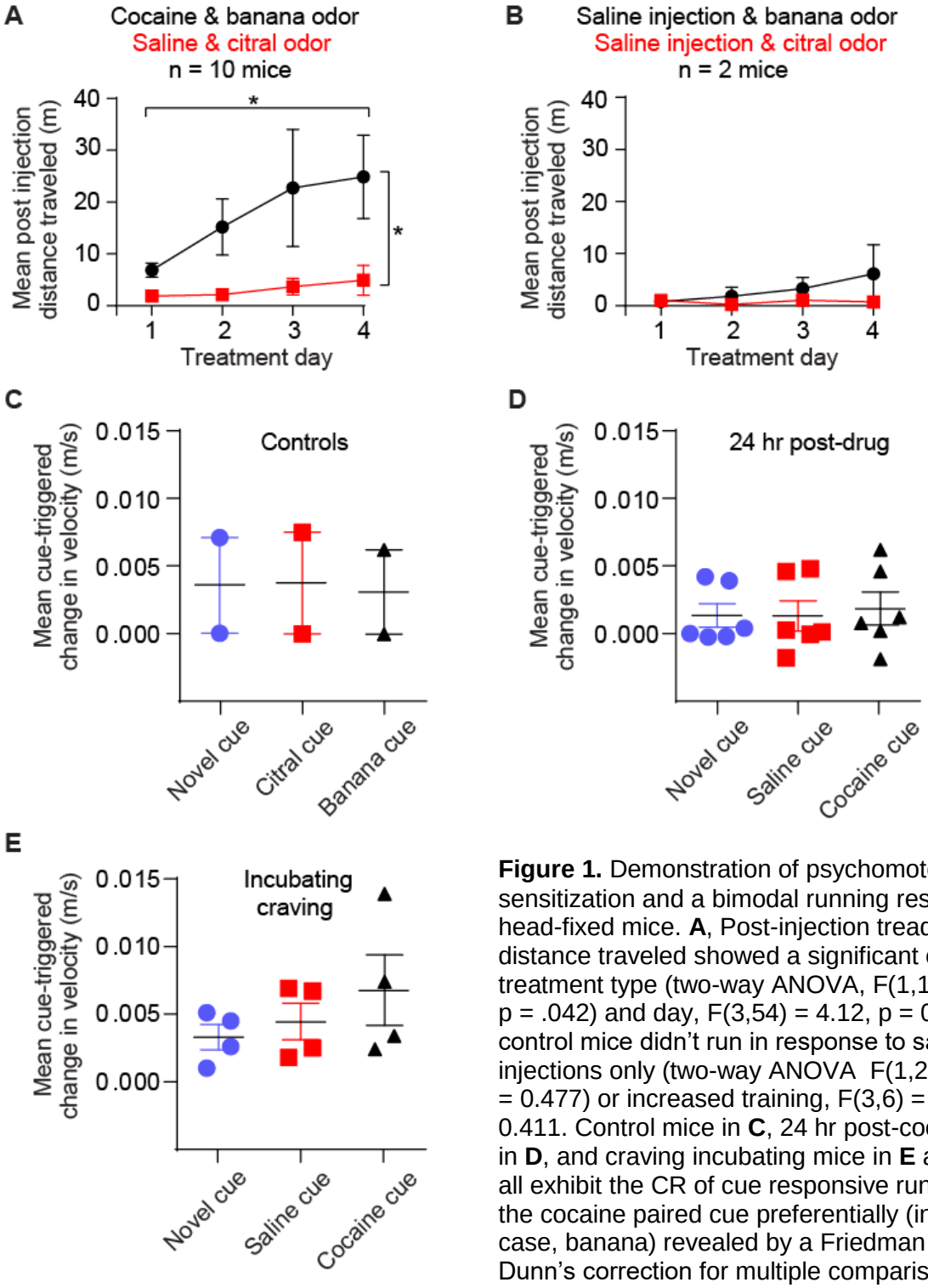
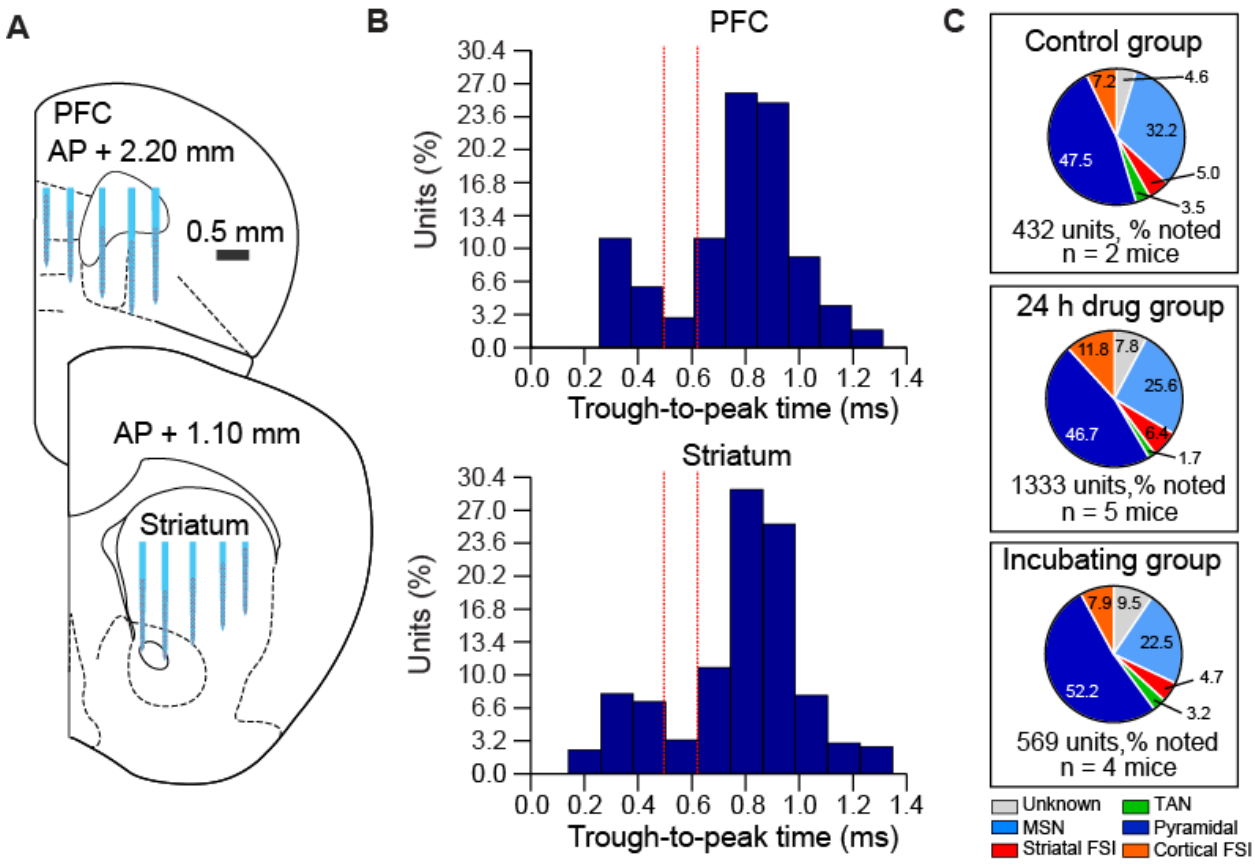


Figure 1. Demonstration of psychomotor sensitization and a bimodal running response in head-fixed mice. **A**, Post-injection treadmill distance traveled showed a significant effect of treatment type (two-way ANOVA, $F(1,18) = 4.77$, $p = .042$) and day, $F(3,54) = 4.12$, $p = 0.011$. **B**, control mice didn't run in response to saline injections only (two-way ANOVA $F(1,2) = 0.75$, $p = 0.477$) or increased training, $F(3,6) = 1.12$, $p = 0.411$. Control mice in **C**, 24 hr post-cocaine mice in **D**, and craving incubating mice in **E** all did not all exhibit the CR of cue responsive running to the cocaine paired cue preferentially (in this case, banana) revealed by a Friedman test with Dunn's correction for multiple comparisons, $p = 0.40$, $p = 0.093$, and $p = 0.431$, respectively. Our results show a bimodal distribution in all movement data, making averages less amenable to study.



D

	Subject										
Type	Ctrl-1	Ctrl-2	24h-1	24h-2	24h-3	24h-4	24h-5	Inc-1	Inc-2	Inc-3	Inc-4
Unknown	13	7	24	8	10	22	40	15	17	11	11
MSN	49	90	59	64	37	61	121	27	29	26	46
FSI	16	6	11	7	7	16	44	6	6	3	12
TAN	6	9	4	6	4	0	9	6	4	4	4
Pyramidal	69	136	114	126	103	155	124	45	123	52	77
Cort. FSI	7	24	15	16	28	69	29	3	13	9	20

Figure 2. Dual-site recording properties. **A**, Insertion sites in all animals with AP coordinates and insertions of electrodes noted to scale. **B**, Histograms of cortical (top) and striatal (bottom) unit trough-to-peak (spike duration) time, with two distributions clearly visible in each region. Red dashed lines denote the classification boundary between FSIs and non-FSI units, or simply interneurons and projection neurons. Units in between are unclassified and not included in subsequent analyses. **C**, Proportion and total number of cells recorded in the each group of mice. **D**, Table indicating subject and total neurons recorded.

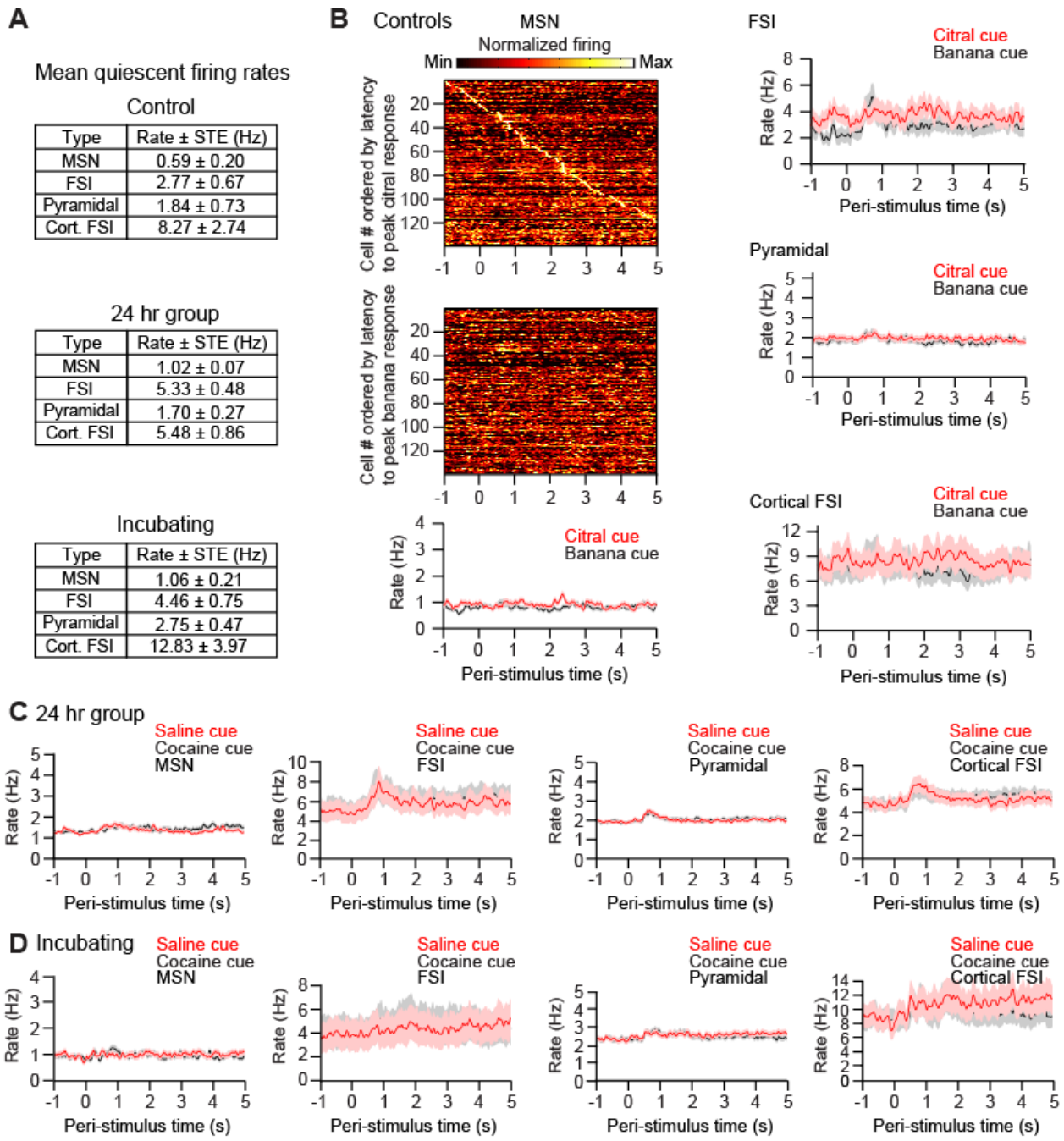


Figure 3. Dual-site recordings after cocaine conditioning fail to yield hypoactivity or cue triggered population responses. **A**, Resting state firing obtained when animals were not moving, prior to cue presentation, yields no significant differences in any condition (Kruskal Wallis ANOVA, all $p > 0.05$), with no cortical hypoactivity in projection cells. **B,** Cue-triggered firing plots of all MSNs in the control condition (left, top & middle) show no distinct pattern of sustained firing for any cues, and when averaged (left, bottom) neither cue elicits a response that differs from 1 s baseline; the same holds true for averaged timeseries of other neuron types in the control condition (right, top through bottom). **C & D,** Both drug groups (24 hr post final injection and incubating craving) share a similar response to control mice with no cue preference in their cell type averaged responses.

CHAPTER 3

FRONTOSTRIATAL CIRCUIT DYNAMICS CORRELATE WITH COCAINE CUE-EVOKED BEHAVIORAL AROUSAL DURING EARLY ABSTINENCE

Abstract

It is thought that frontostriatal circuits play an important role in mediating conditioned behavioral responses to environmental stimuli that were previously encountered during drug administration. However, the neural correlates of conditioned responses to drug-associated cues are not well understood at the level of large populations of simultaneously recorded neurons, or at the level of local field potential (LFP) synchrony in the frontostriatal network. Here we introduce a behavioral assay of conditioned arousal to cocaine cues involving pupillometry in awake head-restrained mice. After just 24 hours of drug abstinence, brief exposures to olfactory stimuli previously paired with cocaine injections led to a transient dilation of the pupil, which was greater than the dilation effect to neutral cues. In contrast, there was no cue-selective change in locomotion as measured by rotation of a circular treadmill. The behavioral assay was combined with simultaneous recordings from dozens of electrophysiologically identified units in the medial prefrontal cortex (mPFC) and ventral striatum (VS). We found significant relationships between cocaine cue-evoked pupil dilation and the proportion of inhibited principal cells in the mPFC and VS. Additionally, LFP coherence analysis revealed a significant correlation between pupillary response and synchrony in the 25-45 Hz frequency band. Together, these results show that pupil dilation is sensitive to drug-associated cues during acute stages of abstinence, and that individual animal differences in this behavioral arousal response can be explained by two complementary measures of frontostriatal network activity.

Keywords: Cocaine, conditioned arousal, large-scale recordings, prefrontal cortex, pupillometry, striatum

Introduction

Environmental stimuli formerly associated with drugs can have a powerful influence on behavior, presenting a long-term risk of relapse after the cessation of drug use (Robinson and Berridge, 1993; O'Brien et al., 1998). This is thought to occur because of functional changes in a variety of brain circuits mediating reward learning, motivation, arousal, and inhibitory behavioral control, which persist in drug abstinence (Everitt and Robbins, 2005; Britt et al., 2012; Munoz-Cuevas et al., 2013; Luthi and Luscher, 2014; MacAskill et al., 2014; Pascoli et al., 2014). The medial prefrontal cortex (mPFC) and ventral striatum (VS) are two interconnected areas, which are believed to play a critical role in these processes (Jentsch and Taylor, 1999; Kalivas et al., 2005; Day and Carelli, 2007; Goldstein and Volkow, 2011). Neuroimaging studies in addicted human subjects have revealed that prefrontal and striatal regions are modulated by cocaine-associated cues (Maas et al., 1998; Childress et al., 1999; Wexler et al., 2001), and that some of these activation patterns correlate with self-reported drug craving (Bonson et al., 2002). Neuroimaging has also revealed alterations in frontostriatal network connectivity following drug use (Wilcox et al., 2011; Hu et al., 2015). However, because of the limited spatiotemporal resolution of neuroimaging techniques, little is known about the neurophysiological correlates of cocaine cue-evoked frontostriatal interactions. At the level of single-neuron measurements, units in the mPFC and VS are known to encode drug-associated cues (Carelli et al., 1993; Chang et al., 2000; Ghitza et al., 2003; Rebec and Sun, 2005; West et al., 2014). However, while individual animal differences in behavioral responses to drugs and their associated stimuli can be significant (Groman et al., 2012; Chen et al., 2013; Munoz-Cuevas et al., 2013; Robinson et al., 2014; Storey et al., 2016) the neural correlates of this interindividual variability are not well understood. Large-scale recordings have the potential to identify novel relationships between neural activity in prefrontal and striatal circuits and drug

cue-evoked behavior, but until now, such measurements have not been widely used together with animal models of addiction.

In addition to eliciting voluntary behavioral responses or subjectively measured craving, drug-associated cues are known to influence autonomic responses, indicating a heightened state of arousal in the form of heart rate and skin conductance changes (Ehrman et al., 1992) and pupil dilation (Rosse et al., 1995). Since the dynamics of prefrontal and other cortical circuits are related to pupil diameter (Aston-Jones and Cohen, 2005; Reimer et al., 2014; McGinley et al., 2015b; Vinck et al., 2015; Joshi et al., 2016),which in turn reflects arousal (Hess and Polt, 1960; Bradley et al., 2008; Vinck et al., 2015), we hypothesized that, during abstinence, drug-associated stimuli cause pupil dilation, that the degree of this effect varies among animals, and that this variability correlates with the dynamics of frontostriatal circuits.

To address this hypothesis, here we simultaneously performed large-scale in vivo neural recordings in the mPFC and VS together with a novel assay of conditioned arousal to cocaine-associated cues in mice in the early stage (first 24 h) of abstinence, which relies on pupil dilation measurements. We found that cocaine-paired olfactory cues induced a stronger pupillary response than saline-paired cues, and that this autonomic response was more selective for cocaine cues than the cue-evoked locomotion response. Large-scale recordings were performed with a customized 512 electrode silicon microprobe (Shobe et al., 2015), providing simultaneous measurements from dozens of electrophysiologically identified neurons in the mPFC and VS, as well as local field potential (LFP) oscillations. We assessed spontaneous and cue-evoked electrophysiological firing properties, and related the level of activation and inhibition to simultaneously measured changes in pupil diameter. We found that the proportion of prefrontal pyramidal cells and striatal projection neurons that were inhibited by cocaine-associated cues was correlated with the degree of pupil dilation. Moreover, we found that 25-45 Hz frontostriatal LFP coherence was correlated with the pupillary response. Together, these

results suggest that suppression of frontostriatal activity, and elevated interregional synchrony, have a related role in triggering arousal to cocaine cues. These measurements provide new insights into the dynamics of frontostriatal circuits when drug-free animals are exposed to stimuli that were previously encountered during drug administration.

Materials and Methods

Animals and surgical procedures

All procedures were approved by the University of California, Los Angeles, Animal Care Committee. We used singly housed male C57BL/6J mice (12–16 weeks old; The Jackson Laboratory), with 10 mice receiving alternating cocaine and saline injections (referred to as the drug group) and 7 mice receiving only saline (referred to as the control group). Animals underwent an initial surgery under isoflurane anesthesia in a stereotactic apparatus to implant stainless steel head restraint bars bilaterally into their skulls using dental cement (Shobe et al., 2015). Animals were anesthetized with isoflurane for a second surgery on the evening prior to the day of the recording session to make a craniotomy over the anterior striatum and mPFC in the right hemisphere. The dura mater was opened to facilitate the insertion of microprobes during the recording session. An additional craniotomy was made over the posterior cerebellum for placement of a silver/silver chloride electrical reference wire. The craniotomies were covered with silicone elastomer sealant (Kwik-Cast, WPI) to seal the exposed skull.

Cocaine conditioning

One week after the first surgery, animals began habituation to head restraint and the behavioral conditioning/testing room for 4 d, with one 30 min session per day. Animals were mounted with the head bar bracket on top of a 200-mm-diameter treadmill ball that was free to rotate forward and backward. No other stimuli were presented during habituation, except for low

background lighting in the enclosed room provided by four 200 lumen LED lamps directed away from the eyes of the mice and toward the walls of the room. The lamps were necessary to prevent a saturation effect in the pupil dilation, which would occur in the absence of any light. After habituation, fully awake head-restrained animals were conditioned over 10 d to injections of cocaine or saline, paired with a previously unfamiliar olfactory cue. Each daily conditioning session for the drug group was devoted to either cocaine or saline, but never both on the same day. The drug group received alternating cocaine (15 mg/kg, 100 µl, s.c.) or saline (100 µl, s.c.) infusions administered over a 1 min period. The infusions were given by means of a syringe pump connected to a 27.5 gauge syringe needle inserted subcutaneously. We removed the needle after each session. To ensure consistency across training and behavioral testing (see next section), we also inserted the needle during the testing session, but it was disconnected from any fluid delivery system. Conditioning started with saline on day 1 and ended with cocaine on day 10. All cocaine or saline administration sessions were paired with a single continuous presentation of odorized air, starting 5 min preinjection and ending 25 min postinjection, when the animal was removed from the conditioning room and returned to its home cage in a vivarium housing facility. Olfactory cues were introduced via an olfactometer by bubbling air (0.15 L/min) through aromatic liquids (citral or isoamyl acetate) diluted 1:10 in mineral oil (Sigma-Aldrich), and mixing this product with a 1.5 L/min stream of air. This mixture flowed through a tube passing 15 mm away from, and perpendicularly to the animal's nose, with a 5 mm opening in the tube aimed at the nose. Half of the drug group animals were counterbalanced and received the opposite drug–odor pairing. The saline group received saline injections during all 10 d of conditioning, paired with alternating citral and isoamyl acetate cues. During each conditioning session, we monitored behavior in the form of treadmill rotation velocity and pupil dilation. Pupillometry video was captured under infrared illumination with the camera (model ACA640, Basler) focused on the right eye of the animal.

Behavioral testing

The craniotomy surgery was performed 7–8 h after the final conditioning session. Behavioral testing and electrophysiological recordings were performed simultaneously in drug-free conditions, in animals abstinent from their last injection of cocaine (drug group) or saline (control group) for 24 ± 3 h. We provided animals a 15 min resting period without any cues, which was used to capture spontaneous activity. Subsequently, we randomly presented the previously paired odors (15 s in length, 30 trials per odor type, with a randomized mean intertrial interval of 20 ± 5 s) while monitoring treadmill locomotion and pupil dilation to assess behavioral responses.

Electrophysiological recordings

A silicon microprobe (Shobe et al., 2015) was assembled targeting the medial prefrontal cortex and ventral striatum (256 electrodes per area, total of 512 electrodes). Each area was targeted with four silicon prongs spaced 0.2 mm apart, each containing 64 electrodes that were arranged in a hexagonal array pattern spanning 1.05 mm along the dorsal–ventral axis. Electrodes had an area of 100 $\mu m^2$and were gold plated (noncyanide gold solution, Sifco) to an impedance of 0.1–0.5 MΩ. In the mPFC, the relatively long span of the electrode array allowed us to simultaneously record from the prelimbic and infralimbic subregions. In the VS, the primary target was the nucleus accumbens core, but, due to the length of the electrode array, we also recorded from the region above the accumbens core (medial striatum). One to two hours prior to recording, the silicon microprobes were coated with a drop of fluorescent dye (DiD, Invitrogen) to assist with histological confirmation of their position. Animals were then mounted on the head restraint system. An electrical reference wire was placed on the cerebellar surface, covered in ACSF-saturated water-absorbing foam (Gelfoam), to improve electrical contact, and sealed with silicone elastomer (Kwik-Cast, WPI). The silicon microprobes were slowly inserted to

stereotactically defined coordinates with a motorized micromanipulator (MP-285, Sutter Instruments). The tip position of the most medial shaft in the mPFC was targeted to 1.90 mm anterior, 0.07 mm lateral, and 3.40 mm ventral relative to bregma in the right hemisphere. The tip of the most medial shaft in the VS was placed in the same hemisphere at 1.0 mm anterior, 0.77 mm lateral, and 4.70 mm ventral relative to bregma. The insertion was monitored with a surgical microscope. After reaching the target depth, a drop of mineral oil was placed on the exposed cortical surface, and a 45 min settling period elapsed before beginning the electrophysiological data acquisition and behavioral testing (see the previous section). We monitored electrophysiological data at a sampling rate of 25 kHz/electrode, together with treadmill ball rotation and olfactory cue delivery time at a sampling rate of 10 kHz. Pupillometry video was synchronously captured at 25 frames/s. Following each recording, the microprobe was cleaned in trypsin solution (Invitrogen), rinsed with deionized water and ethanol, and reused throughout these experiments.

Histology

Coronal brain sections were sliced at 100 μm on a vibratome, and individual sections were placed in order onto a 24 well plate containing ice-cold PBS solution. We then performed immunohistochemistry using standard procedures on the mPFC and striatal sections to determine the placement of the silicon prongs in each region. Sections were stained for neuronal nuclei with chicken-α NeuN primary antibodies (D3S3I, Cell Signaling Technology) and fluorescent α-chicken secondary antibodies (Alexa Fluor 488, Jackson ImmunoResearch). DiD, which diffusively labeled tissue near the probe insertion sites, was used to estimate the final probe position.

Unit classification

Spike sorting was performed using a semi-automated spike waveform template-matching algorithm (Shobe et al., 2015). Each unit was assigned an estimated 3D coordinate as well as a histologically determined brain region. The estimated position coincided with the recording site exhibiting the highest spike amplitude for that unit. Finally, units were classified as principal neurons or interneurons in their respective brain regions. Striatal units with a minimum baseline rate of 0.02 Hz were classified as putative medium spiny projection neurons (MSNs), fast-spiking interneurons (FSIs), or tonically active neurons (TANs), based on spike waveform peak-to-trough width and the coefficient of variation of the baseline firing rate (Bartho et al., 2004; Bakhurin et al., 2016). FSIs were characterized by a narrow spike waveform (maximum width, 0.475 ms). MSNs and TANs both have wider waveforms (minimum width, 0.55 ms; maximum width, 1.25 ms). TANs were separated from MSNs by the regularity of their baseline firing (maximum coefficient of variation, 1.5). Units in the mPFC were classified as putative FSIs or pyramidal neurons using the same spike width separation criterion as described above (Tseng et al., 2006).

Behavioral data analysis

Pupil diameter data were extracted from video frames with the MATLAB image analysis toolbox and custom scripts. To quantify behavioral responses during cocaine or saline conditioning, we calculated the mean postinjection treadmill rotation velocity and pupil diameter per animal. To quantify the cue-evoked behavioral responses during abstinence, for each animal we calculated the change in speed and pupil diameter during the 15 s cue presentation time relative to a 1 s baseline period prior to cue onset. Unless otherwise noted, responses were averaged across all 30 cocaine or saline-paired trials.

Electrophysiological data analysis

The single-unit firing rate was calculated in time steps of 50 ms and smoothed by convolving with a Gaussian kernel (SD = 250 ms). Spontaneous firing activity was obtained by averaging the rate from the first 15 min of the recording, when animals received no olfactory cues and did not move on the treadmill. Cue-evoked activity was obtained by averaging across all cocaine- or saline-paired trials. To determine whether units were excited or inhibited by cues, we performed a permutation test to identify time bins whose firing rate was significantly different from a 1 s baseline period preceding the cue onset (criterion for significance, $p < 0.01$). If two or more consecutive time bins during the cue presentation period (t = 0–15 s from cue onset) were significantly greater (less) than the baseline, the unit was classified as excited (inhibited). Tables 1 and 2 list the number of total, excited, and inhibited pyramidal cells and MSNs per animal in the drug-treated group (n= 10 mice). Partitioning of units into different subregions was done using an objective criterion: all units recorded from the top half of the electrode array were assigned to one partition, and the remaining units were assigned to the other partition. We confirmed histologically that the top and bottom partitions in the mPFC corresponded approximately to the prelimbic and infralimbic cortices, respectively. In the VS, the top and bottom partitions corresponded approximately to the medial striatum and nucleus accumbens core, respectively. Local field potential signals were downsampled off-line to 1 kHz. To quantify the cue-evoked change in LFP coherence, for each animal we calculated the change in 25-45 Hz coherence during the 15 s cue presentation time relative to a 1 s baseline period prior to cue onset. Coherence changes were averaged across all cocaine- or saline-paired trials.

Statistics

Permutation tests to determine significant cue-evoked excitation or inhibition of neural activity were performed with custom Matlab scripts (Shobe et al., 2015). All other statistical

analysis was performed with standard Matlab functions or GraphPad Prism software. ANOVA was followed by Sidak's correction for multiple comparisons. Analysis of neural activity in different electrode partitions was followed by Bonferroni's correction for two comparisons, corresponding to the top and bottom partitions (α was adjusted to 0.025). Correlations between pupillary response and neural activity or synchrony were calculated with both Pearson's and Spearman's correlation coefficients (r and r_s, respectively) to ensure consistency. In all cases, Pearson's and Spearman's correlations were in agreement with regard to the statistical significance of the result. In this edited version of the eNeuro article, as we are omitting the statistical table present in the original article, only Pearson's correlations are reported within the figures.

Results

Conditioned pupil dilation by cocaine-associated cues

We developed a behavioral assay of arousal in response to cocaine-associated cues in fully awake, head-restrained mice (Fig. 1A). Before recording, mice in the drug group (n = 10) were conditioned over a 10 d period with alternating injections of cocaine and saline, which were paired with a specific olfactory cue (Fig. 1B). During this period, mice showed a psychomotor sensitization effect, as marked by increased rotational movement on the treadmill after doses of cocaine (Fig. 1C, left, D; two-way ANOVA: effect of treatment type: $F(1,18) = 31.50$, $p < 0.0001$; effect of treatment day: $F(4,72) = 3.75$, $p = 0.008$; interaction effect: $F(4,72) = 2.64$, $p = 0.041$). At the same time, mice showed increased pupil dilation in response to cocaine injections, which did not significantly change over the course of administration (Fig. 1C, right, E; two-way ANOVA: effect of treatment type: $F(1,18) = 17.66$, $p = 0.0005$; effect of treatment day: $F(4,72) = 0.831$, $p = 0.510$; interaction effect: $F(4,72) = 0.65$, $p = 0.627$).

After conditioning, animals were prepared for concomitant electrophysiological recording and behavioral testing. During behavioral testing, 24 h drug-abstinent animals were randomly exposed to olfactory cues previously associated with either cocaine or saline, but were not given any injections. While some animals moved in response to cues, we found no difference in cue-evoked treadmill rotation velocity between cocaine- and saline-paired odors (Fig. 1F; Wilcoxon matched pairs signed-rank test, $p > 0.99$). In contrast, we found a significantly higher change in pupil diameter following the presentation of cocaine-paired cues (Fig. 1G; Wilcoxon matched pairs signed-rank test, $p = 0.027$). There was no correlation between cue-evoked changes in pupil dilation and locomotion in response to the cocaine-associated odor (Fig. 1H, $r = 0.08$, $p = 0.827$). Figure 1, I and J, shows the time dependence of average velocity and pupil diameter, respectively. Finally, we compared the mean pupillary response of the initial 15 trials and the final 15 trials, and found no consistent change in response to the drug cue (Fig. 1K; Wilcoxon matched pairs signed-rank test, $p = 0.695$) or saline cue (Fig. 1L; Wilcoxon matched pairs signed-rank test, $p = 0.922$), suggesting that elevated arousal in response to drug-associated cues is maintained throughout behavioral testing.

We confirmed our results by repeating conditioning and testing with a group of control animals ($n = 7$) that only received saline injections paired with the same two odors (Fig. 1B). Control animals showed no change in locomotion as a function of either odor type or day of treatment (Fig. 2A; two-way ANOVA, $p > 0.05$ for all effects). Similarly, no changes were observed in pupil diameter during conditioning (Fig. 2B; two-way ANOVA, $p > 0.05$ for all effects). On the test day, we found no statistical difference in either cue-evoked locomotion (Fig. 2C; Wilcoxon matched pairs signed-rank test, $p = 0.297$) or pupil diameter (Fig. 2D; Wilcoxon matched pairs signed-rank test, $p = 0.375$). These results show that during short-term abstinence, cocaine-associated olfactory cues selectively increase behavioral arousal in the form of pupil dilation, but do not selectively influence locomotion.

Cocaine does not alter average spontaneous and cue-evoked neural dynamics early in abstinence

To monitor frontostriatal network dynamics in abstinent drug-conditioned animals, we developed a 512 electrode silicon microprobe enabling large-scale simultaneous recordings in the mPFC and VS (Fig. 3A). Correct electrode placement was confirmed histologically (Fig. 3B). Units in each area were classified as principal cells (pyramidal cells in the mPFC, and MSNs in the VS) or interneurons based on spike waveform width (see Materials and Methods; Fig. 3C). We recorded a total of 2063 single units from drug-treated mice and 1141 units from control mice. In drug-treated mice, 72.7% of mPFC neurons fit our criteria for being pyramidal neurons, 14.7% were putative FSIs, and 12.6% were unclassified. In drug-treated mice, 63.4% of VS neurons fit our criteria for MSNs, 18.5% were putative FSIs, 6.4% were putative TANs, and 11.7% were unclassified (Fig 3D). In control mice, 72.3% of mPFC neurons fit our criteria for being pyramidal neurons, 10.5% were putative FSIs, and 17.2% were unclassified. In control mice, 60% of VS neurons fit our criteria for MSNs, 20.8% were putative FSIs, 7.2% were putative TANs, and 12% were unclassified.

Cocaine exposure is frequently associated with prefrontal hypoactivity (Trantham et al., 2002; Chen et al., 2013). Furthermore, the prelimbic and infralimbic regions of the mPFC are often associated with opposing roles in cocaine-seeking behavior (Peters et al., 2008). To examine spontaneous firing properties in these subregions, we selected units from the top and bottom halves of the electrode array (Fig. 4A, cortex, B, striatum), and compared the mean spontaneous firing rate per animal of different neuronal populations between the drug and control groups. After correcting for two comparisons, we did not find a significant difference in the spontaneous firing rate of cocaine-treated animals in any of the cortical and striatal subregions examined (Fig. 4C–F; Mann–Whitney U test, $p > 0.025$, none of the results are

significant after adjusting α to 0.025 following Bonferroni's multiple comparisons correction). When comparing the mean spontaneous rate per animal, the relatively small number (n = 3–5) of FSIs recorded in certain subregions of some animals may have contributed to the large variability of our FSI results, making it challenging to detect an effect of cocaine. Therefore, we also performed a comparison after pooling all FSIs in the drug and control groups. Again, we found no significant difference in firing rate between these groups (Fig. 4G,H; Kolmogorov–Smirnov test, $p > 0.05$). These results demonstrate that for the areas targeted with our electrodes, cocaine does not significantly alter the spontaneous cortical or striatal activity of principal neurons and FSIs early in abstinence.

We next examined cue-evoked neural activity. A portion of principal neurons in the mPFC and VS showed significant excitation or inhibition in response to cocaine-associated olfactory stimuli (Fig. 5A–D), demonstrating that neural activity is modulated by olfactory cues. However, there was no difference in the fraction of neurons that was excited or inhibited by cocaine versus saline cues (Fig. 5E–H; all $p > 0.05$, paired Wilcoxon signed-rank test). These results show that, on average, these circuits do not appear to preferentially encode cocaine-associated cues.

Conditioned pupillary response correlates with frontostriatal inhibition

Since we did not find differences in the mean level of cocaine- and saline-associated cue encoding in the mPFC and VS, we next tested whether the variability in behavioral responses among individual drug-experienced animals (n = 10) could account for the observed patterns of neural activity. Since drug-paired cues selectively affected pupillary responses, but not treadmill locomotion, we focused on individual animal changes in pupil diameter in response to cocaine cue presentation. We found that the mean change in pupil diameter was unrelated to the fraction of significantly excited pyramidal neurons (Fig. 6A, $r = 0.213$, $p = 0.554$) and MSNs (Fig.

6B, r = 0.371, p = 0.292). In contrast, there was a significant positive correlation between pupil diameter change and the fraction of inhibited pyramidal neurons (Fig. 6C, r = 0.809, p = 0.005) and MSNs (Fig. 6D, r = 0.779, p = 0.008). These results were obtained by combining units from all electrode depths in the mPFC and VS. To examine whether these relationships are consistent within different cortical and striatal subregions, in each area we partitioned units into two groups according to their location on the electrode, as previously described (see Materials and Methods; Fig. 4A,B). In the mPFC, the fraction of inhibited units in both the top and bottom halves of the electrode array, corresponding approximately to the prelimbic and infralimbic cortex, maintained a relationship between the fraction of inhibited cells and the change in pupil diameter (Fig. 6E, r = 0.905, p = 0.0003; Fig. 6F, r = 0.743, p = 0.014). In the VS, the fraction of units recorded from the top half of electrodes (corresponding to the medial striatum) showed no correlation to behavior (Fig. 6G, r = 0.571, p = 0.085), whereas the fraction of units from the bottom half (corresponding to the nucleus accumbens core) inhibited by the drug cue remained highly correlated to pupillary response (Fig. 6H, r = 0.839, p = 0.002). These results suggest that during early drug abstinence, inhibition of the medial prefrontal cortex and the nucleus accumbens core is involved in conditioned arousal to drug-associated cues. In contrast, medial striatal MSNs do not appear to have such a relationship with behavior.

We next looked for correlations between the fraction of excited or inhibited cells, and treadmill running evoked by cocaine-paired cues. There was no significant correlation (Fig. 7A–D, p > 0.05; exact probability values are in the figure legend), suggesting that frontostriatal activity is more related to conditioned arousal in the form of pupil dilation than it is to conditioned locomotion.

To determine whether neural activity has any relationship with saline cue-evoked pupil dilation, we also looked for correlations between the fraction of excited or inhibited cells and pupil dilation in response to saline-paired cues. There was no statistically significant correlation

in any of these comparisons (Fig. 7E–H, $p > 0.05$). Thus, while we found significant correlations between cocaine cue-evoked frontostriatal inhibition and pupil dilation, there was no corresponding relationship of saline cue-evoked activity with pupil dilation. This is consistent with brain circuit alterations following drug exposure that selectively mediate conditioned arousal responses to drug-associated stimuli, but not neutral stimuli.

Conditioned pupillary response correlates with frontostriatal LFP coherence

Since the mPFC projects to the VS (Berendse et al., 1992; Voorn et al., 2004), neural activity in these areas is related (Chang et al., 2000; Ishikawa et al., 2008). To gain insight into the significance of this interaction for our behavioral task, we examined whether neural synchrony in the form of LFP coherence was related to cue-evoked arousal measured via pupillary responses. LFP signals were measured together with single-unit action potentials on the same electrodes. Frontostriatal LFP coherence spectra revealed a peak in coupling between the mPFC and VS at ~25-45 Hz both under resting conditions (Fig. 8A) and during cocaine cue presentations (Fig. 8B). These rhythms are within the frequency range of LFP signals that were previously found to be sensitive to cocaine administration (McCracken and Grace, 2013). Across the animals tested in the drug group ($n = 10$), we found a significant correlation between the mean change in cocaine cue-evoked 25–45 Hz coherence and pupil diameter (Fig. 8C, $r = 0.801$, $p = 0.005$). This relationship held even after partitioning LFP signals from the top and bottom half of the electrode array in each area (results not shown; $p < 0.025$, all correlations remain significant after adjusting α to 0.025 following Bonferroni's correction for two comparisons). Finally, we did not find an analogous relationship between the change in saline cue-evoked LFP coherence and pupil diameter (Fig. 8D, $r = 0.467$, $p = 0.172$). As shown in Figure 7E–H, this is consistent with a conditioned arousal response to drug-associated stimuli, but not neutral stimuli.

Discussion

This study introduced an assay of behavioral responses to cocaine-associated olfactory stimuli, which was specifically developed to support large-scale electrophysiological recordings in awake head-restrained mice. The assay used measurements of pupil dilation and treadmill movement. Pupillary response is an autonomic behavior that is modulated by the state of arousal of an animal (Hess and Polt, 1960; Bradley et al., 2008; Vinck et al., 2015). Pupil dilation is influenced by locomotion but can also be modulated independent of movement (Vinck et al., 2015), providing two related but separable approaches for tracking how mice respond to cocaine injection or cocaine-associated cues. Specifically, as drug conditioning progressed animals responded to cocaine injections with increased locomotion on a circular treadmill, consistent with a psychomotor sensitization effect (Robinson and Berridge, 2008). Pupil dilation was also impacted by cocaine administration, but this response did not change significantly as drug conditioning progressed, which may reflect a ceiling effect after just the first injection. After a 24 h drug abstinence period, there was greater pupil dilation in response to odors previously paired with cocaine than with saline, whereas there was no difference in locomotion on the treadmill. This demonstrates that under the conditions of our behavioral test, pupillary response is more sensitive to cocaine cues than locomotion, at least in the early stage of drug abstinence. The most parsimonious interpretation of these results is that animals are more aroused by odors previously paired with cocaine because of an associative learning process that occurred during the conditioning period (O'Brien et al., 1998). We therefore presume that interindividual variations in the amplitude of the pupil dilation effect reflect differences in conditioned arousal. An open question of this study is whether cue-evoked pupil dilation is related to elevated drug craving. Analogous effects have been shown in studies with addicted human subjects using other autonomic responses (heart rate and skin conductance) (Ehrman et al., 1992). Thus, while

we cannot be certain that the mice in this study craved drugs, the conditioned arousal response appears to be consistent with elevated craving.

To the best of our knowledge, our study is the first use of pupillometry to assess responses to drug-paired cues in mice. The rapid occurrence of the dilation effect (within 24 h after the last drug administration) presents a sensitive, noninvasive method to measure drug cue arousal in models of addiction. Furthermore, since pupillometry is compatible with neuroimaging methods in human subjects (Bray et al., 2008), the task introduced here has potentially useful applications in human addiction research and diagnosis.

We combined this behavioral assay with silicon microprobe technology to simultaneously record from dozens of electrophysiologically identified prefrontal and striatal units. We initially focused on differences in spontaneous neural activity between drug- and control-treated mice 24 h after the last cocaine injection. We found that spontaneous activity in the areas examined was unaltered. Cocaine use frequently has been associated with prefrontal hypoactivity (Jentsch and Taylor, 1999; Trantham et al., 2002; Sun and Rebec, 2006; Chen et al., 2013). From our data, we infer that hypoactivity is not a strong feature of the circuits we examined in the first 24 h of cocaine abstinence. In fact, we even noted a tentative trend toward higher pyramidal cell firing in the infralimbic portion of the PFC (Fig. 4C; Mann–Whitney U test, $p = 0.033$, which is not significant after adjusting α to 0.025 following Bonferroni's correction for two comparisons), suggesting that short-term and long-term states of abstinence may be characterized by different states of resting brain activity. An additional consideration is that the electrophysiological recordings and spike sorting are inherently biased toward more spontaneously active neurons. Thus, it is possible that this analysis did not take into account relatively silent units.

We also examined cue-evoked neural activity, focusing on units with significant excitatory or inhibitory responses. Our results show that, on average, cocaine and saline cue-

evoked frontostriatal network dynamics were not discernibly different in the early stage of drug abstinence. Previous studies suggest a somewhat complex activation pattern involving a mixture of enhanced and reduced activity in prefrontal and striatal networks by drug-associated cues (Ghitza et al., 2003; Goldstein and Volkow, 2011; Mahler and Aston-Jones, 2012; West et al., 2014). Several factors may contribute to our observed level of cue-modulated neural activity in the frontostriatal network. The first consideration is that the relatively short duration of abstinence in our study (24 h) does not induce significant incubation effects (Pickens et al., 2011). An increase in cue-evoked VS neural firing has been observed after extended (30 d) periods of abstinence (Hollander and Carelli, 2007; West et al., 2014). A second factor may be that the recording sites in the VS targeted regions that do not discriminately encode cocaine from neutral cues (Ghitza et al., 2003).

While neither the mPFC nor the VS selectively encoded cocaine cues on average, we noticed a substantial variability in the neural response between individual animals. We therefore tested whether these results could be explained by variations in the behavioral responses of individual animals. We found that changes in pupil diameter in response to cocaine-paired odors were significantly correlated with the overall fraction of inhibited principal cells in the mPFC (including both prelimbic and infralimbic areas) and the nucleus accumbens core. At the same time, we found that the fraction of excited units was not predictive of behavior. These results show that inactivation (or active suppression) of medial prefrontal and ventral striatal output is related to an elevated autonomic response to cocaine-paired stimuli.

Noradrenergic signaling from the locus coeruleus is strongly implicated in behavioral arousal (Aston-Jones and Cohen, 2005). The locus coeruleus is reciprocally connected with the cortex (Chandler et al., 2014; Schwarz et al., 2015), providing a possible route for mediating the observed relationship between pupil diameter and neural activity. The potential importance of these reciprocal interactions is supported by findings of prefrontal modulation of locus coeruleus

activity (Sara and Herve-Minvielle, 1995; Jodo et al., 1998), and electrophysiological studies showing relationships between cortical dynamics and pupil dilation (Reimer et al., 2014; McGinley et al., 2015a; Vinck et al., 2015; Joshi et al., 2016). In addition to noradrenergic input, frontostriatal circuits are likely to be modulated by dopaminergic signaling (Everitt and Robbins, 2005; Goldstein and Volkow, 2011).

It is worth noting that neural inhibition in both the prelimbic and infralimbic aspects of the mPFC exhibited a significant relationship with pupil dilation. This suggests that the suppression of activity in these regions is linked to higher levels of arousal in response to cocaine cues. This finding may appear to be at odds with studies showing opposing roles for these regions in cocaine seeking, with the prelimbic and infralimbic cortex respectively driving and suppressing this behavior (Peters et al., 2008; LaLumiere et al., 2010). On the other hand, there is also evidence of a less dichotomous role of these regions in drug and natural reward-related behavior (Moorman et al., 2014; Moorman and Aston-Jones, 2015). Furthermore, it is possible that due to the considerable functional diversity of the prefrontal cortex (Fuster, 2001; Pessoa, 2008), this circuit differentially controls autonomic and voluntary responses to drug-associated cues.

The mPFC projects to the VS (Berendse et al., 1992), which in turn can indirectly influence cortical activity via mesocorticolimbic feedback loops (Everitt and Robbins, 2005). We observed coherent LFP oscillations between the mPFC and VS, which are thought to synchronize VS activity to afferent signals from the mPFC (McCracken and Grace, 2013). The strength of this coherence in the 25-45 Hz frequency band was found to be correlated with the degree of cocaine cue-evoked pupil dilation, suggesting that the observed neural dynamics in the mPFC and VS were related to frontostriatal network interactions.

Together, this study used large-scale neural recordings in mice to examine neurophysiological properties related to drug abstinence The high throughput of these

measurements combined with a novel behavioral assay of cocaine cue arousal, enabled us to identify new relationships between frontostriatal network dynamics and behavioral responses to cocaine-associated stimuli. Our finding that cue-evoked pupil dilation is related to lower activity in the mPFC and VS, as well as increased LFP coherence between these structures, may provide new insights for understanding and preventing drug relapse. These results also highlight the importance of accounting for interindividual behavioral variability in studies of addiction, which can be significant (Groman et al., 2012; Chen et al., 2013; Munoz-Cuevas et al., 2013; Storey et al., 2016). Finally, the compatibility of our behavioral task with awake head-restrained mice creates new opportunities for studying systems-level neural dynamics in rodent models of addiction using a variety of complementary recording and brain circuit dissection tools.

Figures

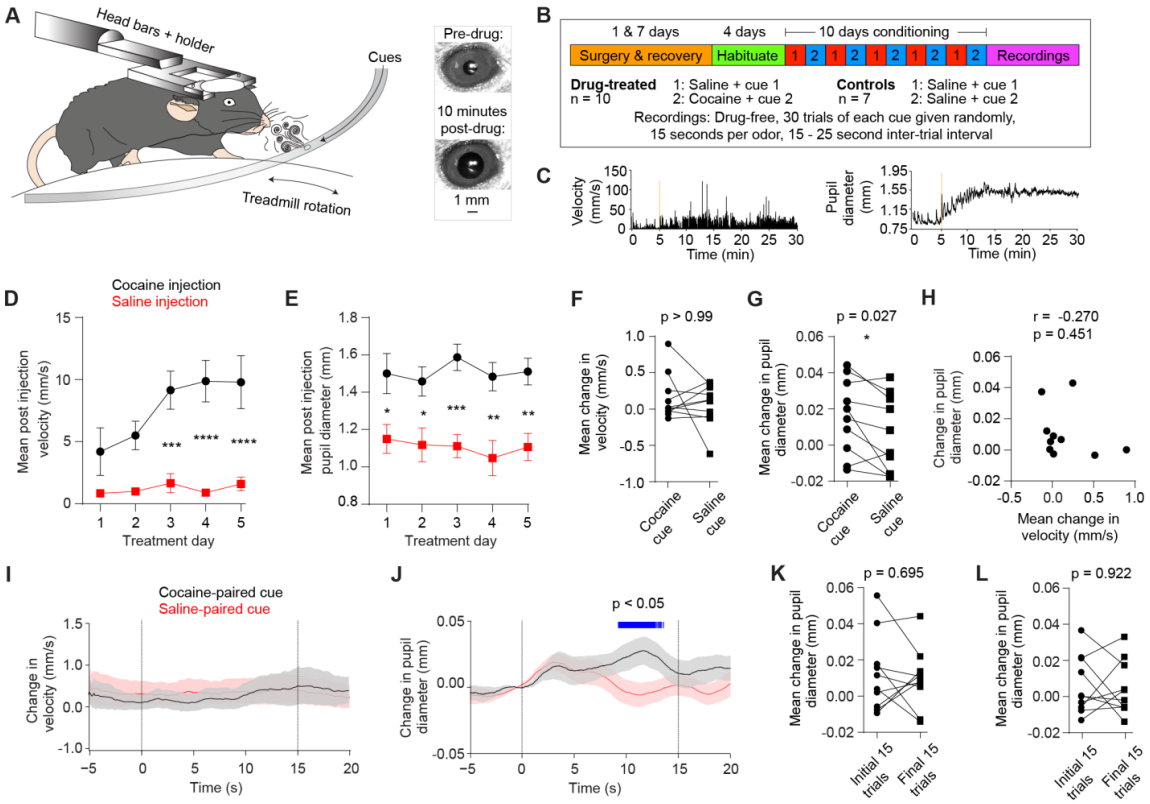


Figure 1. Enhancement of cocaine cue-evoked pupil dilation during drug abstinence. **A**, Left, Setup for drug conditioning using olfactory cues in head-restrained mice. Behavior was monitored in the form of pupil dilation and circular treadmill rotation velocity. Right, Image of the pupil of an animal before drug injection (top) or 10 min after a single injection of cocaine during conditioning (bottom). **B**, Timeline of surgery, habituation, drug conditioning, and recording. **C**, Time dependence of absolute value of treadmill rotation velocity (left) and pupil diameter (right) for one representative animal on the first day of cocaine conditioning. Dashed line indicates the time of drug injection. **D**, For the drug group (n = 10), post-injection treadmill rotation velocity showed a significant effect of treatment type (two-way ANOVA, $F_{(1,18)} = 31.50$, $p < 0.0001$) and day of treatment ($F_{(4,72)} = 3.75$, $p = 0.008$). **E**, Post-injection pupil diameter showed a significant effect of treatment type (two-way ANOVA, $F_{(1,18)} = 17.66$, $p = 0.0005$), while there was no effect of day of treatment ($F_{(4,72)} = 0.831$, $p = 0.510$). **F**, When presented with the cues on the test day in the absence of cocaine, drug-experienced mice (n = 10) showed an equal locomotor response to cocaine- and saline-associated cues (Wilcoxon matched-pairs signed rank test, $p > 0.99$). **G**, Drug-experienced mice showed a higher pupil dilation in response to cocaine-associated cues (Wilcoxon matched-pairs signed rank test, $p = 0.027$). **H**, Cocaine cue-mediated pupil dilation changes were not correlated with treadmill rotation changes ($r = 0.08$, $p = 0.827$). **I**, Cue-triggered treadmill rotation velocity vs time averaged across drug-treated mice shows no difference in locomotion between cocaine- and saline-paired cues. Dashed lines indicate cue onset and offset. **J**, Mean cue-triggered change in pupil dilation vs time. Blue lines indicate time bins where the cocaine and saline cues elicited significantly different dilation (paired t test, $p < 0.05$). **K**, There was no significant difference in pupillary response between the initial 15 trials and final 15 trials of the cocaine cue (Wilcoxon matched pairs signed-rank test, $p = 0.695$). **L**, There was no significant difference in pupillary response between the initial 15 trials and the final 15 trials of the saline cue (Wilcoxon matched pairs signed-rank test, $p = 0.922$). Data in **D**, **E**, **I**, and **J** are reported as the mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$.

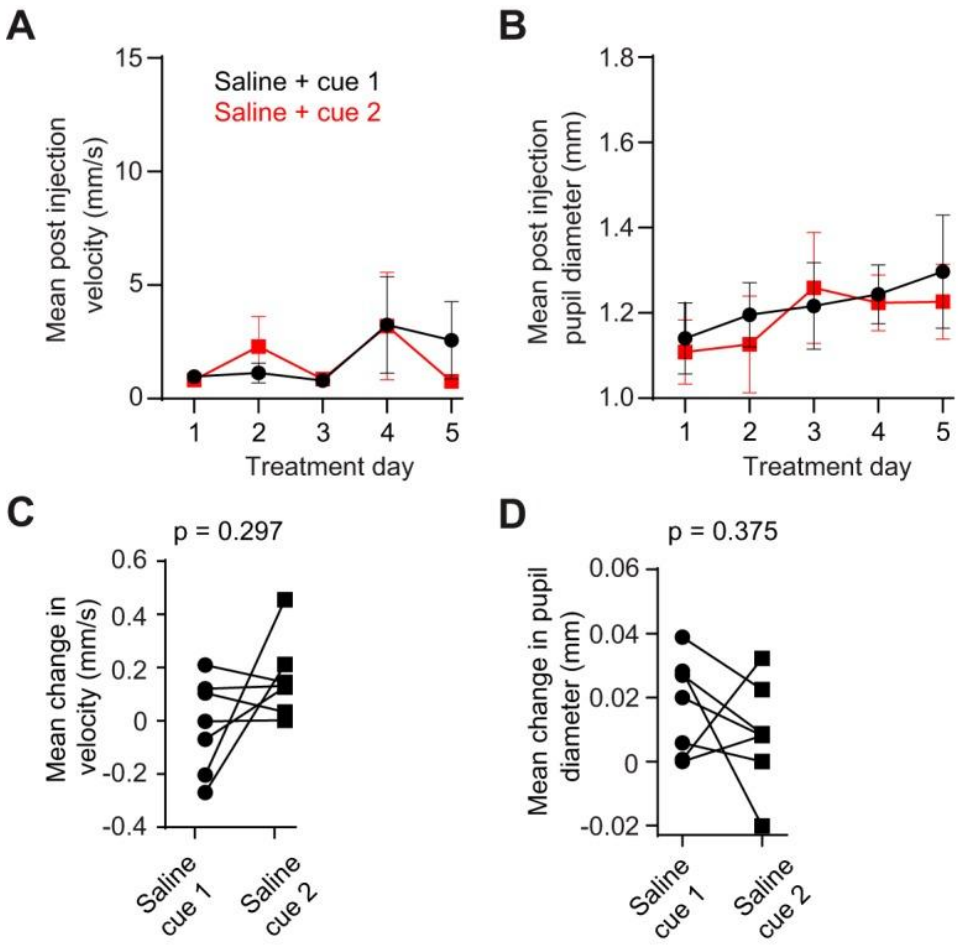


Figure 2. Control mice do not show differences in cue-evoked pupil dilation. **A**, For the control group (n = 7), postinjection treadmill rotation velocity showed no effect of odor type (two-way ANOVA, $F_{(1,12)}$ = 0.024, p = 0.878) or day of treatment ($F_{(4,48)}$ = 1.450, p = 0.232). **B**, Control group animals showed no effect of odor type ($F_{(1,12)}$ = 0.072, p = 0.794) or day of treatment ($F_{(4,48)}$ = 1.777, p = 0.149). **C**, When presented with cues on the test day, control group mice (n = 7) showed an equal locomotor response to both cues (Wilcoxon matched-pairs signed rank test, p = 0.297). **D**, When presented with cues on the test day, control group mice showed an equal pupillary response to both cues (Wilcoxon matched-pairs signed rank test, p = 0.375). Data in **A** and **B** are reported as the mean ± SEM.

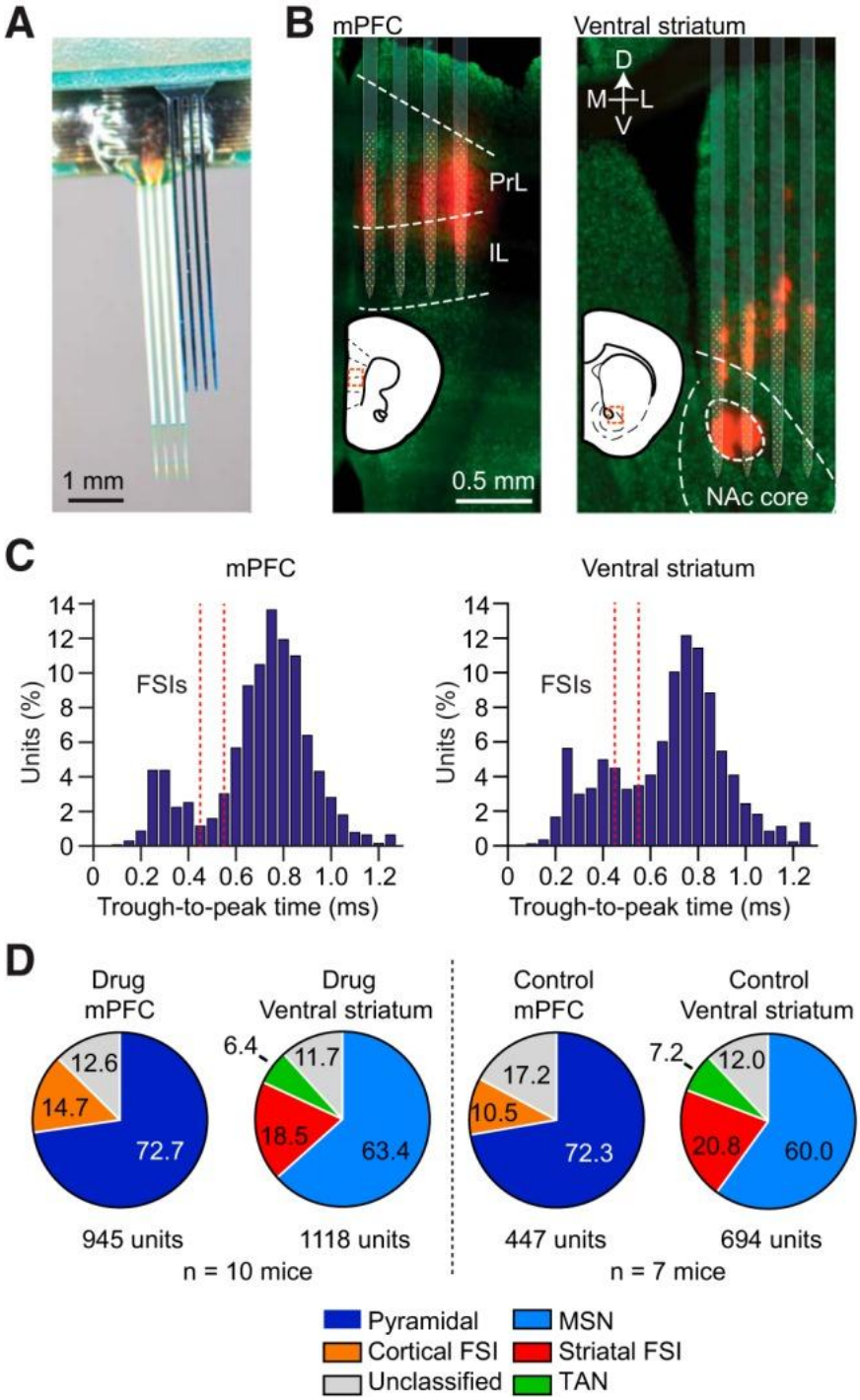


Figure 3. Large-scale neural recordings in the frontostriatal circuit. **A**, A 512 electrode silicon microprobe targeting the mPFC and VS. **B**, Representative reconstruction of electrode placement in the mPFC (left) and VS (right) from the same animal. Red, DiD dye; green, NeuN staining. **C**, Distribution of trough-to-peak spike duration in the mPFC and VS. Red dashed lines denote the classification boundary between FSI and non-FSI units (units between the lines are unclassified). **D**, Proportion and total number of cells recorded in the mPFC and VS in the drug and control groups.

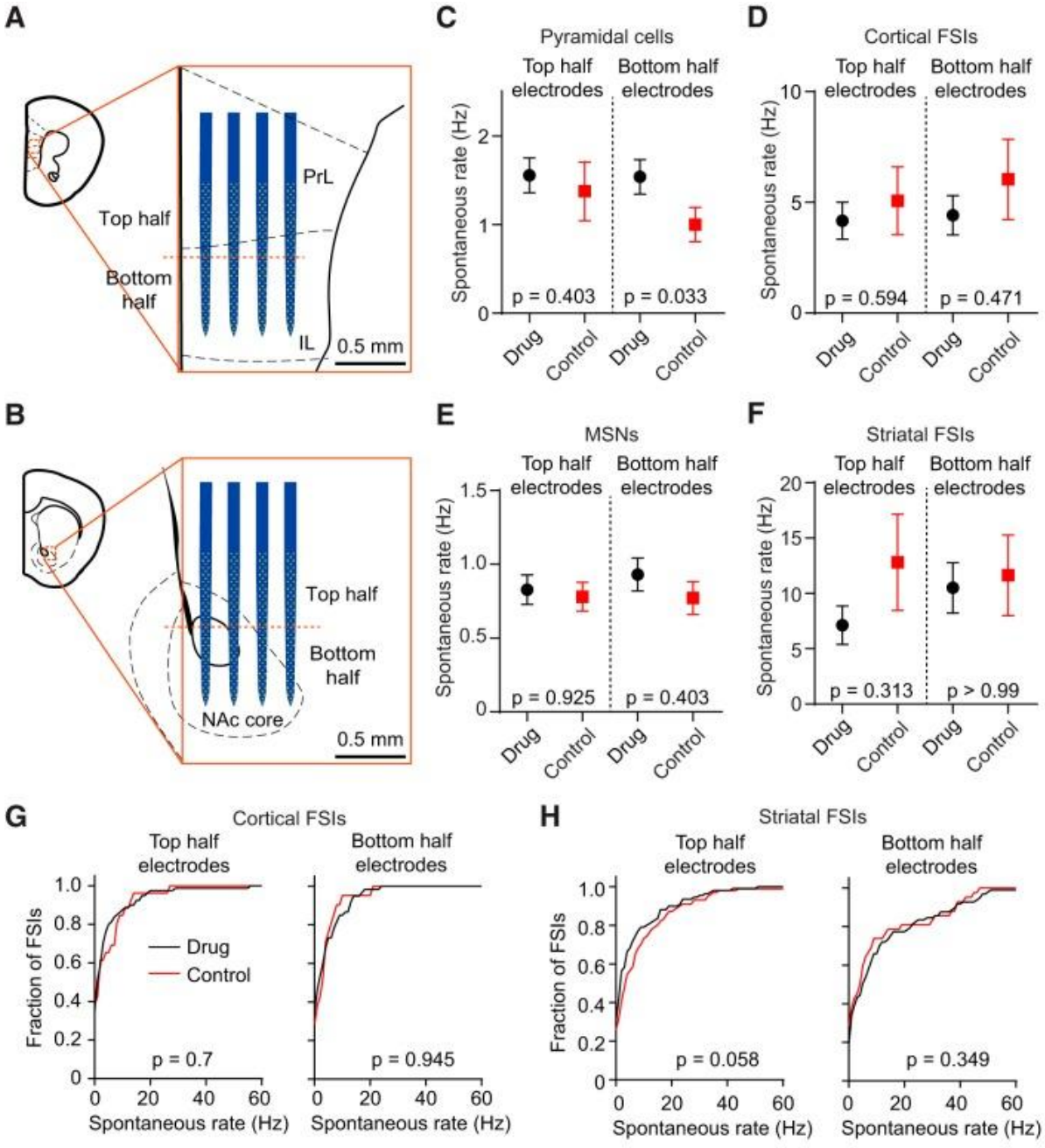


Figure 4. Cocaine does not alter spontaneous cortical or striatal firing rate in early abstinence. **A**, **B**, Illustration of how units in the mPFC and VS were partitioned according to their location on the top or bottom half of the electrode array. **C–F**, There was no difference between the average firing rate of principal cells or FSIs in any of the subregions examined (Mann–Whitney *U* test, $p > 0.025$; exact probability values are listed in the figure, α was adjusted to 0.025 after Bonferroni's correction for two comparisons, corresponding to the top and bottom partitions). Data in **C–F** are reported as the mean ± SEM firing rate across the animals in the drug ($n = 10$) and control ($n = 7$) groups. **G**, **H**, There was no difference in the pooled spontaneous FSI firing rate in any of the subregions examined (Kolmogorov–Smirnov test, $p > 0.05$; exact probability values are listed in the figure).

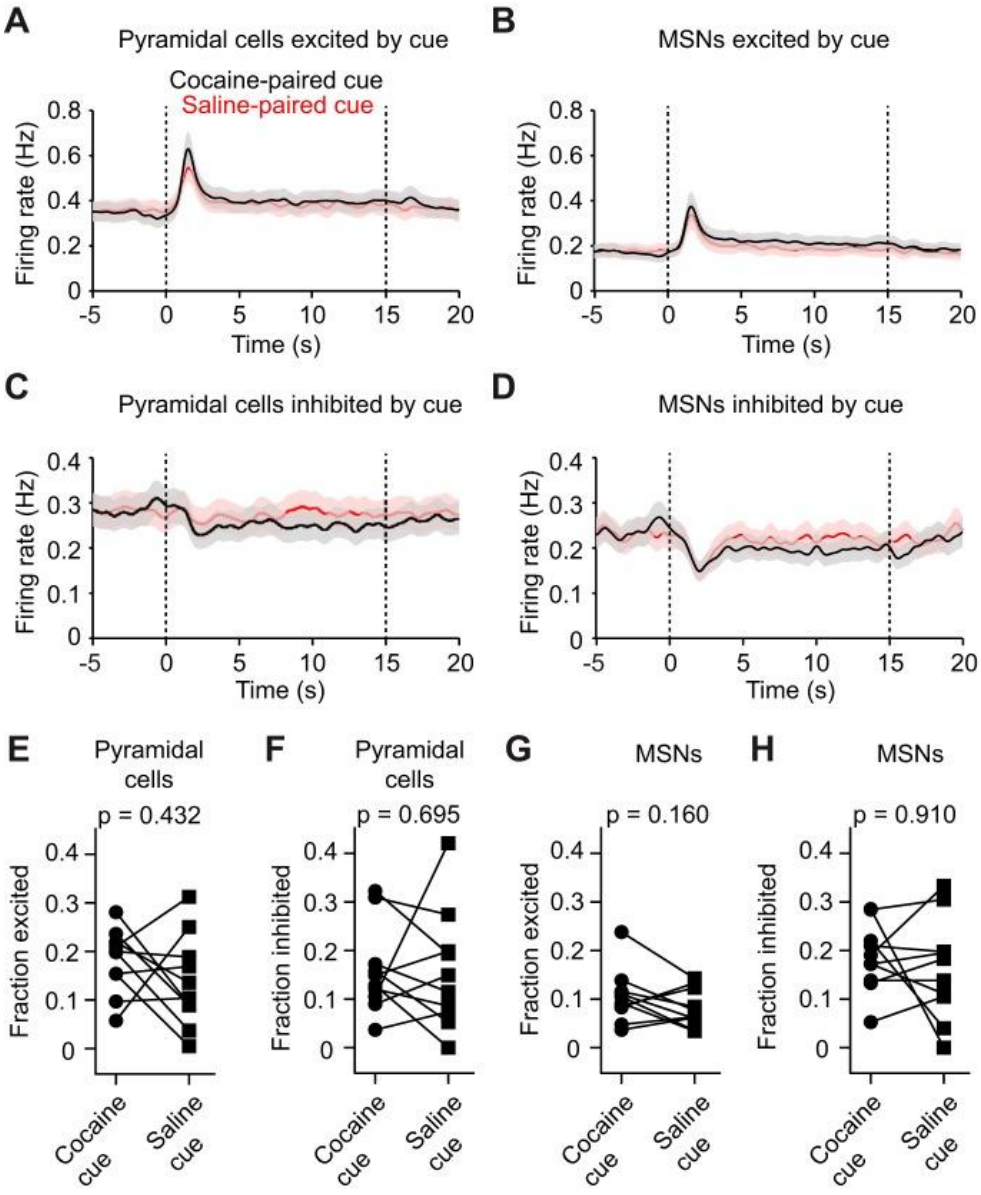


Figure 5. Average cue-evoked activity is not selective for cocaine-associated stimuli. **A**, **B**, Mean cue-triggered firing rate of all significantly excited pyramidal cells and MSNs, combined across data from the drug treatment group. Black and red lines, respectively, indicate the response to cocaine- and saline-paired cues. Dashed lines indicate cue onset and offset. There was no significant difference between the response to cocaine- and saline-paired cues at any time point (paired permutation test, $p > 0.05$). **C**, **D**, Mean cue-triggered firing rate of all significantly inhibited pyramidal cells and MSNs, combined across data from the drug treatment group. There was no significant difference between the response to cocaine- and saline-paired cues at any time point (paired permutation test, $p > 0.01$). Data in **A–D** are reported as the mean ± SEM. **E**, **F**, There was no difference in the average fraction of cortical pyramidal cells excited or inhibited by cocaine cues (Wilcoxon matched pairs signed-rank test, $p > 0.05$). **G**, **H**, There was no difference in the average fraction of MSNs excited or inhibited by cocaine cues (Wilcoxon matched pairs signed-rank test, $p > 0.05$). Data in **E–H** denote the fraction of cells per animal in the drug group ($n = 10$).

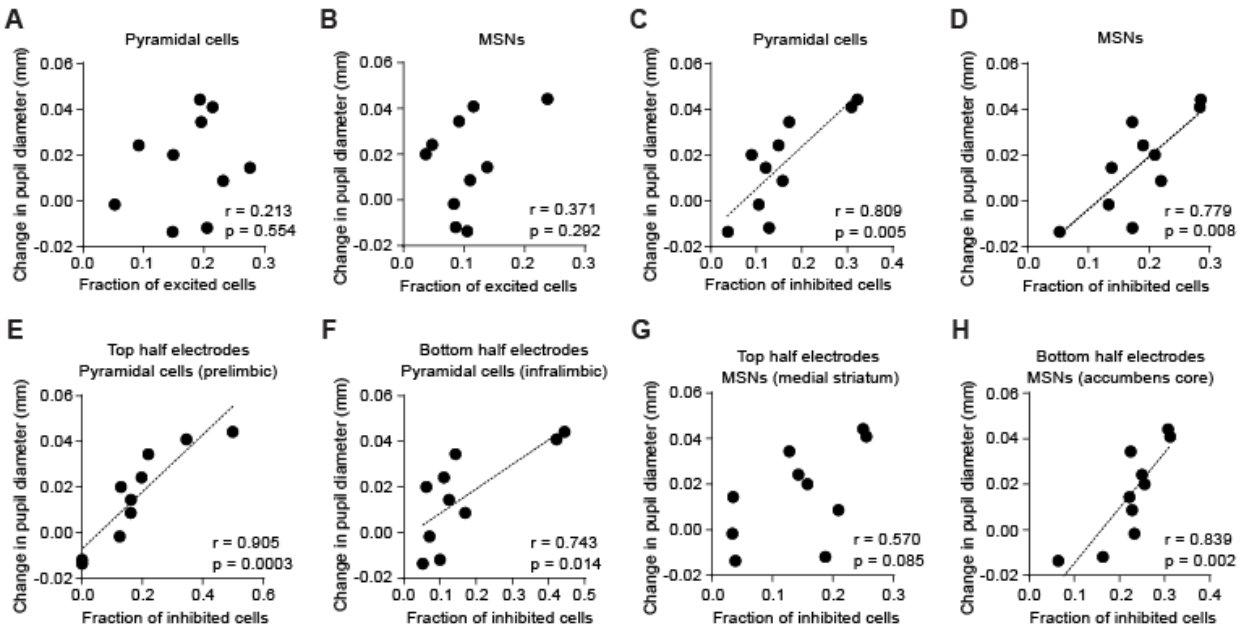


Figure 6. Cocaine cue-evoked pupil dilation correlates with frontostriatal inhibition. **A**, There was no correlation of mean change in pupillary response to the fraction of cortical pyramidal neurons excited by the cocaine-paired cue (r = 0.213, p = 0.554). **B**, There was no correlation of mean change in pupillary response to the fraction of MSNs excited by the cocaine-paired cue (r = 0.371, p = 0.292). **C**, The mean change in pupillary response was correlated with the fraction of inhibited pyramidal neurons (r = 0.809, p = 0.005). **D**, The mean change in pupillary response was correlated with the fraction of inhibited MSNs (r = 0.779, p = 0.008). Results from **A–D** were obtained by combining cortical or striatal principal cells across the entire electrode array. **E**, **F**, Inhibited units in both the prelimbic and infralimbic cortex maintained their relationship to pupillary response (Pearson's correlation, $p < 0.025$; α was adjusted to 0.025 after Bonferroni's correction for two comparisons; exact probability values are listed in the figure). **G**, Inhibited MSNs in the medial striatum were uncorrelated to pupillary response (r = 0.570, p = 0.085). **H**, Inhibited MSNs in the nucleus accumbens core were correlated with pupillary response (r = 0.839, p = 0.002). Plots show data from the drug group (n = 10).

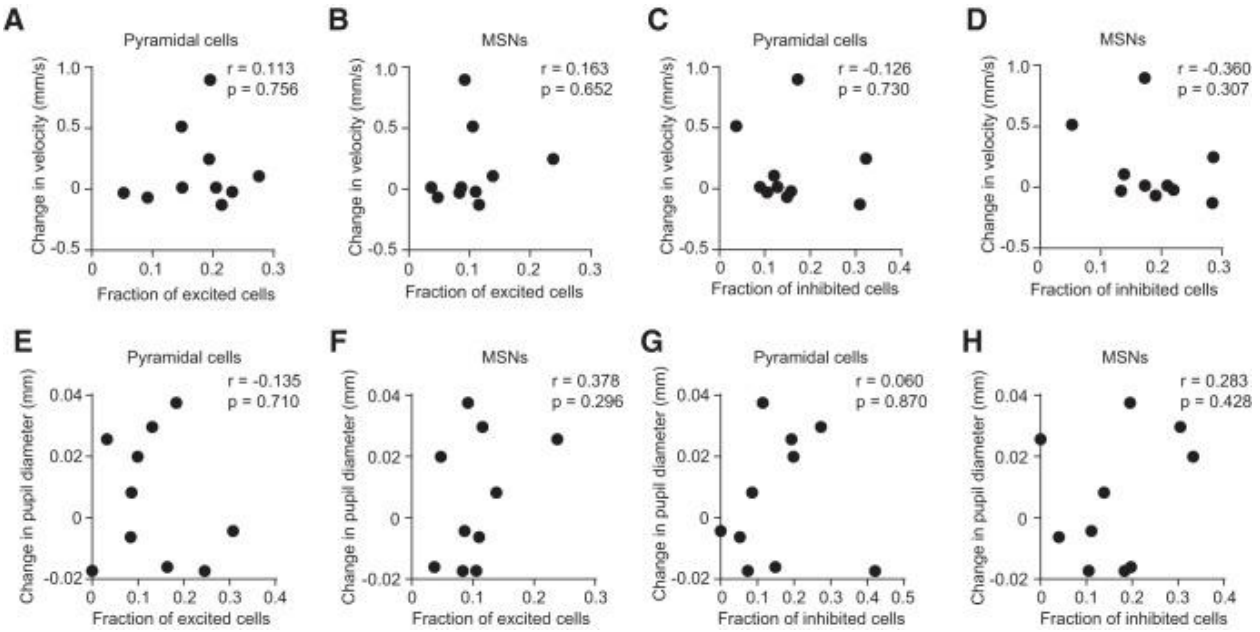


Figure 7. No correlation of neural activity with cocaine cue-evoked treadmill speed or saline cue-evoked pupil dilation. **A**, There was no correlation of the mean change in treadmill velocity with the fraction of cortical pyramidal neurons excited by the cocaine-paired cue (r = 0.113, p = 0.756). **B**, There was no correlation of the mean change in treadmill velocity with the fraction of MSNs excited by the cocaine-paired cue (r= 0.163, p = 0.652). **C**, There was no correlation of the mean change in treadmill velocity with the fraction of cortical pyramidal neurons inhibited by the cocaine-paired cue (r = −0.126, p = 0.73). **D**, There was no correlation of the mean change in treadmill velocity with the fraction of MSNs inhibited by the cocaine-paired cue (r = −0.36, p = 0.307). **E**, There was no correlation of the mean change in pupillary response with the fraction of cortical pyramidal neurons excited by the saline-paired cue (r = −0.135, p = 0.701). **F**, There was no correlation of the mean change in pupillary response with the fraction of MSNs excited by the saline-paired cue (r = 0.367, p = 0.296). **G**, There was no correlation of the mean change in pupillary response with the fraction of cortical pyramidal neurons inhibited by the saline-paired cue (r = 0.060, p = 0.869). **H**, There was no correlation of the mean change in pupillary response with the fraction of MSNs inhibited by the saline-paired cue (r = 0.283, p = 0.428). Results from **A–H** were obtained by combining cortical or striatal principal cells across the entire electrode array. Plots show data from the drug group (n = 10).

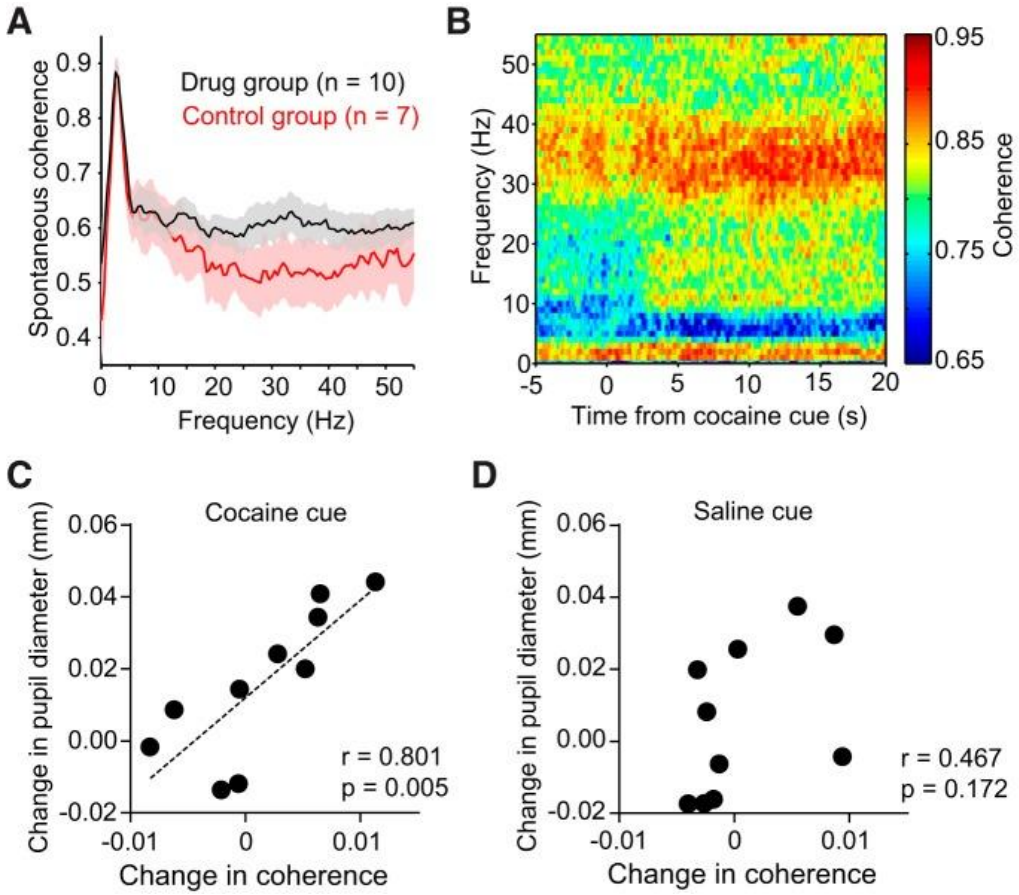


Figure 8. Cocaine cue-evoked pupil dilation correlates with 25–45 Hz frontostriatal LFP coherence. **A**, Frontostriatal coherence spectra of spontaneous LFP activity. Black and red lines, respectively, indicate the mean ± SEM of spectra recorded from animals in the drug (*n* = 10) and control (*n* = 7) groups. Note the peak at ~25–45 Hz for the cocaine group. **B**, LFP coherence spectrogram from one animal showing modulation of coherence by olfactory stimuli. Data are aligned to cocaine cue onset. **C**, The mean change in pupillary response was correlated with the change in 25–45 Hz LFP coherence during the presentation of cocaine cues (r = 0.801, p = 0.005). **D**, The mean change in pupillary response was uncorrelated with the change in 25–45 Hz LFP coherence during the presentation of saline cues (r = 0.467, p = 0.172).

CHAPTER 4

HALLMARKS OF NEURAL CRITICALITY AND UNIVERSALITY IN A MODEL OF EARLY ABSTINENCE FROM COCAINE

Abstract

Self-organized criticality is a hallmark of complex dynamic systems perched at phase transitions. It is thought to be an integral part of natural complex processes, and is an attractive concept in neuroscience due to how systems that operate at or near criticality possess scale-free statistical properties and fluctuations. This universal scaling may be a basis through which miniscule synaptic events may scale up to whole-brain circuits in providing adaptive cognitive function, which may be held in common with other systems with markedly different physical manifestations. Much is yet to be understood about prefrontal cortical and non-cortical systems *in vivo* in the context of SOC, in both the healthy brain as well as in disease states such as addiction, as drug exposure may alter the critical state's attractor and tuning to be a source of drug-seeking and cognitive dysfunction. Here we've developed a novel toolset in order to characterize SOC in our previous mPFC/striatum dataset and assessed how cocaine alters SOC dynamics *in vivo* at rest. In the cortex of cocaine-dosed mice, we found that critical tuning is enhanced compared to controls and that the degree of tuning at rest correlates with pupillary dilation to both cues. Additionally, system complexity is enhanced in drugged mice, but is only correlated to criticality in controls. These results are consistent with literature supporting SOC as a fundamental emergent property of cognition. This is the first study to examine how drugs affect brain states in terms of criticality, and is also consistent with our previous report on interindividual variability. It appears that cocaine may shift the brain towards criticality in a manner dependent on the state of the network, a heretofore unreported aspect of drug abuse.

Keywords: Cocaine, self-organized criticality, neural avalanches, large-scale recordings, prefrontal cortex, striatum

Introduction

The world is predictably dangerous at best and unpredictably dangerous at its worst, and so the brain is the most complex biological system evolved to handle survival in such a world. An emergent property of clusters of neurons, self-organized criticality (SOC) may be the most fundamental and parsimonious expression (Fiser et al., 2010; Berkes et al., 2011) of a neural network to respond to changes and perturbations in a scale-free manner (Fiser et al., 2010; Berkes et al., 2011), allowing for high dynamic range in sensory responses and increased complexity in encoding of information (Cocchi et al., 2017). The end result is that the brain, being an interacting, multi-scale network, benefits from this emergent property and can guide adaptive or potentially addiction-fueled, maladaptive behaviors (Hesse and Gross, 2014; Shriki and Yellin, 2016).

SOC potentially is the framework that unites descriptions of interacting systems and could be universal to all manner of natural observations and systems that are flexible and distributed among many components across time and space: systems such as earthquakes, wildfires, birds flocking, magnetic fields inside ferromagnets, and even thought experiments in predator-prey interactions (Berryman, 1992; Sethna et al., 2001; Markovic and Gros, 2014; Virkar and Clauset, 2014; Cocchi et al., 2017). Indeed, one of the most appealing aspects of studying criticality is that it allows for such comparisons between physical phenomena across dramatically different scales and markedly diverse phenomena, a so-called “universal scaling” function to aid in understanding complexity, or systems with many parts (Stanley, 1999). Even within the brain, activity occurs across many different scales simultaneously, from subcellular processes that aid in plasticity, to the circuit that responds and reorganizes a part of a behavior and memory, and finally with the whole brain enabling a behavior and perpetuating survival. We must be wary though, as although a vast consilience of evidence supports the existence of criticality as a mathematical concept and observable aspect of systems in physics, the addition

of criticality as an explanation for a variety of computational problems and metaphysics of mental phenomena remains to be tested rigorously (Timme et al., 2016).

In order to describe how SOC may be important in cognition, we set forth an explanation of each of the important aspects of criticality. First, criticality can be addressed in simple and complex systems, but they must be dynamic and consist of interacting, similar components that exist at bifurcations or phase transitions (Bak, 1990; Vano et al., 2006; Beggs and Timme, 2012). SOC refers to criticality that has no external tuning provided, rather, it is emergent from the complex system that supports it, through exertion of energy and an intrinsic "memory" of that exertion, as the system recovers resources or resets changed variables (Bak et al., 1990; Beggs and Plenz, 2003). SOC systems have scale-free fluctuations typically referred to as "avalanches" that are highly susceptible to noise within the system (Cocchi et al., 2017), which, no matter their size or power, are statistically distributed following a power law, best seen in log-log coordinates. As power laws have no characteristic scale and their fit lines have the same slope everywhere (hence, scale-free), their data follow a fractal structure and are self-similar at any scale, meaning any and all avalanches are self-similar in shape and follow a universal scaling function.

Real spatiotemporal data exist at the smallest to the largest scales of phenomena that demonstrate criticality, for example, the Ising model of magnetism. Experiments (Sethna et al., 2001) can demonstrate the Ising model (McCoy and Wu, 1973), in which varying the temperature of a ferromagnet changes the spin state of each lattice site's electrons. These electrons influence their neighbors to align their spin to the same direction (NN or nearest neighbor interactions), and with little added energy in the form of heat, a strong net magnetization occurs, as all the spins will point in the same direction. This would be a phase with excess coordination but no fluctuation, important to note for later. With lots of added thermal energy, NN interactions in spin alignment lose out to the random spins influenced by the

heat, yielding no net magnetization; in this case, the phase has excess fluctuation but no coordination of spins. When the system is tuned to the critical temperature, NN interactions and stochastic flips due to heat counter each other and discrete spatiotemporal domains both large and small emerge where spins are pointed up *and* down, across the medium. It can be said that between any two lattice sites, spin coordination and fluctuation, dubbed the dynamic correlation (DC), are maximal at this point and 0 at the other two extremes (Beggs and Timme, 2012). The average DC will of course reach 0 if lattices are further and further away, and its maximum distance before reaching 0 can be given by Γ. If plotted in log-log coordinates, the slope of DC / distance follows a power law, typically with a negative exponent close to the golden ratio. At the critical point then, Γ is maximal, as a stochastic spin flip can then influence neighbor upon neighbor through space and time across the medium giving rise to the aforementioned domains, referred to as an avalanche of activity or communication (Beggs and Plenz, 2004).

In this case communication requires both coordination *and* fluctuation in the system state, as information transfer itself only occurs when there is uncertainty in the system, as is explained by a simplified definition of information entropy (Shannon, 1948; Corominas-Murtra et al., 2014). In a simple analogy, there is no net communication if all NN interactions are making each lattice have a positive spin, as there is no uncertainty in this system at low temperature. At temperatures far above the critical temperature, there is too great uncertainty and little coordination for there to be information transfer or integration, and thus, communication. System entropy and complexity are summarized in a recent paper demonstrating neural pseudodata with an openly available criticality and complexity toolbox, where complexity may be thought of as this simplified communication integration analogy (Marshall et al., 2016). It is at the critical temperature in this example that communication or integration is maximized.

At criticality, as mentioned before, avalanches or fluctuations in data (whatever the system's state variable may be) and their correlations are scale-free and are described as a

power law, rather than exponential, a property referred to as “slowing down” (Fraiman et al., 2009; de Arcangelis et al., 2014). At this point during a phase transition, fluctuations in state are the largest (having great magnitude in their variability) and slowest (their decay is slow), rather than small and weak. Recalling the example of spin correlation length, those domains of correlated spins can span the entire ferromagnet or be very small, and this also coincides with a marked increase in the autocorrelation function of the system (McAteer et al., 2016). Next, these scale-free system-state data are by nature self-similar and when within a spatio-temporal system, their shape, when collapsed, are fractal (Sethna, 2006; Friedman et al., 2012). These events, which may be the actual sounds from a thesis being crumpled up, populations of animals, magnetic spin correlation length (Barkhausen noise), earthquake magnitude, or even avalanche size (sandpiles and neural bursts, particularly), when plotted as a single timeseries, can sound like crackling noise when played through a speaker, hence the name (Perkovic et al., 1995). Essentially, the deformation of a system elicits response events that have a broad range of sizes, both in magnitude and duration. The power-law exponent for the log-log fit of event magnitude or correlation length vs. frequency is typically described by a negative golden ratio, no matter the phenomenon.

Importantly, criticality confers computational advantages like information complexity (Tononi et al., 1994; Timme et al., 2016), high information storage and transmission capacity for efficient communication (Shew et al., 2011), and high dynamic range and sensitivity for dynamic switching and tuning up of weak inputs to provide information to entire systems (Kinouchi and Copelli, 2006). Although we previously mentioned how communication or information entropy is optimized at criticality, it is not a leap to understand that dynamic range and sensitivity would be enhanced at a phase transition. When a stochastic spin flip in the Ising model occurs, it has a potential to change the shape of entire domains through avalanches of finite NN interactions. On the other hand, the issue of information complexity and integration in criticality can be

understood through another example using modeling data using cellular automata (CA) (Li et al., 1990). CA are commonly used in “game of life” simulations, where under looping iterations, artificial cells can replicate or die under certain rules, and their generation time is manipulated along with an interaction parameter. CA innately form coherent oscillatory structures, or unpatterned chaotic structures at varying interaction parameters, but at a critical interaction setting can exhibit mixtures of both, at what is dubbed the “edge of chaos” (Langton, 1990). At this point, structures may span the entire virtual surface and repeat coherent patterns over a long time, yet be susceptible to minute changes that can have long lasting impacts on repeated structures. It takes an unusually large number of generations for the system to leave this state and enter a wholly chaotic or wholly oscillatory mode. The presence of long lasting structure that allows dynamics and change to be carried within larger patterns is characteristic of criticality and complexity. In a sense, the entropy of the system is able to be moved through fractal avalanches across scales, not necessarily stuck in perpetuating a single oscillating pattern or whole divergence into chaos (Tononi et al., 1994).

The evidence for these phenomena in neural and psychophysical/psychobiological systems is mounting, with multiple reviews and books on neural data demonstrating power laws published within the last decade. Although SOC remains debated in neuroscience’s subfields, sufficient hypothesis testing has yielded enough data to demonstrate more than just bare principles. More than a decade ago, it was seen that *in vitro* neocortical cultures grown on top of electrode plates, when recorded for hours at a time, exhibit power law behaviors in their firing patterns (Beggs and Plenz, 2003, 2004). These are of course the aforementioned avalanches, which have a characteristic recording array-wide activity profile, bounded by brief periods of inactivity. Since that time, additional i*n vitro* murine data from alternate brain regions as well as *in vivo* primate physiology and human fMRI data have been collected, all consistent with aspects of criticality (Eguiluz et al., 2005; Petermann et al., 2009; Haimovici et al., 2013). The

tuning of neural slices towards and away from criticality via pharmacological manipulations, inferred by the changing of avalanche distribution from exponential to a power law, has also revealed a maximum information transfer and dynamic range of response to weak inputs at the critical point (Stewart and Plenz, 2006; Shew et al., 2009; Shew et al., 2011), and similar experiments have shown a similar principle in vivo in rats that awaken from anesthesia.(Gautam et al., 2015) Human imaging data corroborates this and has shown loss of critical dynamics concomitant with propofol-induced loss of consciousness (Tagliazucchi et al., 2016). More recent experiments have demonstrated that maximum mutual information is achieved in awake mouse cortex at criticality (Fagerholm et al., 2016). At the behavioral level, Stevens's power laws can explain how animals can respond to stimuli efficiently across several orders of magnitude (Stevens, 1957), and modeling data from olfactory glomeruli recordings have shown that a wide dynamic range of response is achieved when this sensory system operates at a critical state (Kinouchi and Copelli, 2006). Clearly, a comprehensive review of neurobiological observations consistent with criticality is outside the scope of this thesis, given the vast data that supports the criticality hypothesis (Boonstra et al., 2013; Cocchi et al., 2017).

It is assumed that in order to observe power laws in nature, data must not be undersampled (Priesemann et al., 2009), as the network observed has to have as many interacting components observed as possible, meaning increasing both channel count and recording duration is imperative. Additionally, close electrode spacing is required for any adjacent neurons that participate in the same avalanche to be detected in the array together (Ribeiro et al., 2010). In the literature, a commercially available 96-electrode electrophysiological array (Blackrock Microsystems, Inc., Salt Lake City, UT, USA) was thought to have close enough electrode spacing with a 400 μm inter-electrode gap, but was shown to be unable infer criticality *in vivo* (Dehghani et al., 2012), while some other arrays with larger spacing have been able to (Klaus et al., 2011). Our previously collected data used 256

electrodes in cortex and 256 in striatum distributed evenly across 4 shanks separated by 200 μm each, with an inter-electrode distance on each shank < 40 μm. Post-hoc assessment of criticality in our data is justified, as our equipment and timescale of recording are well within previous experimental parameters from other groups that were used to observe criticality.

Until this thesis, hallmarks of criticality were yet to be observed in the striatum and deep prefrontal cortex *in vivo* in murine models, despite already being seen in vivo in superficial cortical layers through imaging methods, electrode arrays, and slice physiology (Karimipanah, 2016) . We are uniquely poised to leverage our advanced recording technology to observe hallmarks of criticality in our datasets. By showing that the striatum and one of its crucial executive inputs operate at criticality, we can aid in understanding neural correlates of decision making and errors therein due to addiction, as well as provide a framework for understanding the already extensive depth of electrophysiology data within the basal ganglia network. Not only that, but by virtue of finding hallmarks of criticality using a novel experimental prep and recording apparatus *in vivo*, we further legitimize the neural criticality hypothesis. Unexpectedly, level of focus in mindful meditation, a key element of behavioral therapy for sufferers of PTSD and depression (Hayes et al., 2011), was recently found to correlate with critical brain states through EEG methods (Irrmischer et al., 2018). By assessing critical dynamics in addicts, we may develop protocols for therapy and behavioral interventions that are tailored for each patient and their focus. Not only that, but future applications of high-throughput data may be to test and refine our understanding of the importance of criticality and information entropy in brain states and learning, ultimately to understand the basics of neurocognition and even the development of a neuromorphic computer or true artificial intelligence (Avizienis et al., 2012; Bose, 2017).

Interestingly, although criticality is thought to be an integral part of consciousness, sensory response, and attention as outlined above, there is nothing yet known about how markers of criticality are affected by drugs of abuse. After all, systemically taken drugs of abuse

do change qualia and perception in the short and long term, a condition called psychosis(Baker, 1989; Kristensen, 1994; Stankewicz and Salen, 2018), and the craving state contributes to dysphoria (Gardner, 2011), and symptoms of withdrawal have consequences on perception and cognition across brain regions. Although we cannot measure conscious subjective experiences in mice, we can observe changes in the systems-level features of criticality as correlates of an aspect of consciousness.

Here we describe previously established criteria (Friedman et al., 2012) for an avalanche in both regions, and in our results we ensure consistency with other groups' data as well as test for differences in resting-state avalanche properties between our prior drug-treated and drug-free mice. These properties include the log-log fit exponent or alpha (α), goodness of log-log fit or coefficient of determination (R^2), and a novel metric that serves as a proxy for self-similarity in avalanches, the median absolute error (MAE) universality score of mean collapse shapes. We also apply simple quantitative metrics to correlation matrices of collapse shapes to corroborate our universality metric. We expected our resting state cortical data in the drug treated group to have the most self-similar or fractal avalanches, as indicated by how tuned their universality metrics are towards criticality, compared to their control counterparts. This is due to assumptions in how, mathematically, the slight increase in resting firing we previously saw at rest in cocaine-dosed cortices may boost the system's complexity (Tononi et al., 1994). Understandably, this is counterintuitive to how criticality is thought to influence cognition, attention, and awareness, given how substance abusers are stereotyped to have lower cognitive faculties (Ahern et al., 2007). Animals that had the greatest degree of inhibition and pupil dilation during the task would be pushed away from the critical state a concomitant amount in the task, as these would be consistent with current understanding of activity vs. resting state dynamics of critical brain networks (Cocchi et al., 2013; Hearne et al., 2015). This would mean that peak criticality at rest would likely correlate to a large pupil dilation, as these mice would be

primed for a strong, beneficial stimulus-response. Additionally, since complexity is thought to be enhanced at the critical state, we tested for differences in a measure of complexity in our groups, as well as correlated complexity to critical tuning, as new works suggest that critical tuning maximizes complexity concomitantly (Marshall et al., 2016; Timme et al., 2016). Finally, since the striatum has not yet been observed in the context of neural criticality, observing these markers in a part of the basal ganglia represents a novel application of this toolset with potential to serve as a marker for severity of addiction and potential for relapse.

Materials and Methods

As these analyses are based on Chapter 3, no further animals were sacrificed for these post-hoc tests. 10 mice were part of the cocaine-treated group and 7 were part of the saline-only group, and all behavioral testing, electrophysiology, and electrophysiology data processing were unchanged aside from the following added neural avalanche processing methods.

Neural avalanche criteria and statistical testing of universal critical dynamics

In both brain regions, we adapted criteria established by Friedman, et al. as these were sufficient to observe universal critical dynamics in cortical slice culture (Friedman et al., 2012). Avalanches in each brain region were defined as bouts of activity in 5 ms bins as small as single spikes, up to bouts consisting of hundreds of units, bounded by 5 ms of inactivity (Fig. 1). The single brain region limitation is due to our ability to detect spikes occurring on either 256 channel probe on our 512 channel array. We selected only projection cells and fast-spiking interneurons in either region to work with, as in our previous research. We collected all avalanches in either region per animal both at rest before the task and during the behavioral task. Avalanches were plotted on a log-log plot of avalanche size vs. count and duration vs. count in Matlab, with a power law fit made per animal, with a corresponding fit exponent α (Fig. 2). Fit exponents were

subject to rank-based tests to assess difference in slope between treatment groups during rest. Only resting periods were used in any avalanche analyses, as post-cue periods would have to be concatenated to enable a long enough period to analyze, and doing so breaks avalanches and treats early cues the same as late cues in terms of potential composition and encoding. To test for universality in our data, unlike other groups (Friedman et al., 2012), we used all avalanche durations more than 25 ms long that had more than 19 avalanches in them; this is a more strict data requirement for universality, as we are not arbitrarily limiting which data to show. In other words, to observe universality, we utilize as much avalanche data as is reasonably amenable to plotting, as opposed to only a handful of avalanche durations. In particular, these durations are common enough to allow for reproducible statistical results, while avalanches below 25 ms long are extremely variable and those with fewer than 20 samples tend to be extremely long and variable. If our recordings were longer, a wider variety of avalanche durations would be picked, but with a tradeoff of recording stability, given recordings were performed acutely in awake-behaving mice. Using z-scores, we then collapsed the mean shape of each of those avalanches that were within the defined durations and plotted the z-score of spikes per bin over the fraction of avalanche duration in each animal in each activity and treatment condition (Fig. 3B). As it has previously shown that cortical avalanches that collapse well do not collapse at all if spike times are jittered, even by 30 ms (Friedman et al., 2012), we did not jitter spike times to test for significance in that way. Each set of shapes was then assessed for similarity to each other through interpolating shapes per animal with 100 points per shape in MATLAB and finding the median absolute error (MAE, Fig. 4) of all collapse shapes; this was obtained as a final variable representing fractal fit or best estimate of universality. We call this unitless (z-score based) variable the MAE universality score, and as it decreases the error between avalanche shapes decreases, and thus, indicates more self-similarity. We used these universality data in ranked statistical tests as before to observe differences among our

drug and control groups in both regions (Fig. 4 & Fig. 5). All statistical tests were rank-based, unless otherwise indicated, due to the sample size in each group and inability to match samples (10 drugged mice vs. 7 controls). These data were plotted with previously collected pupil data and subjected to Spearman's ranked correlation tests to assess how criticality is associated with behavioral data representing a learned association to a drug during early stages of abstinence, with the test statistic being r_s (Fig. 5). To address additional mathematical concerns regarding a new metric for self-similarity, we created correlation matrices of collapse shapes for each animal (cortex-rest only shown in Fig. 6A & 6B) and compared counts of all significant positive Pearson's correlations in the drug-treated group vs. the control group, as a broad method of examining self-similarity (Fig. 7). We also correlated collapse counts to pupillary dilation, again assessing how criticality is associated with behavioral data and how consistent our measures are with each other. Finally, we utilized system-state entropy based test for complexity in our crackling noise data (Marshall et al., 2016) in each animal at rest, per region, and compared complexity between treatment groups and correlated complexity to our own MAE universality metric (Fig. 8. & Fig. 9).

Results

Demonstration of scale-free crackling noise and avalanche shapes in vivo

As critical or scale-free phenomena follow power laws and exhibit crackling noise (as an example of scale-free crackling noise data, see Fig. 1A, top), we first decided to examine all firing in each brain region per animal to show crackling noise as a proof-of-concept. If our probes were unable to demonstrate this very general phenomenon, then, generally, they would be unable to show more fine features of criticality. We took all cortical and striatal spiking data for each animal in each condition (rest and cue-responsive) in 5 ms bins and plotted binned firing over time (Fig. 1A, bottom, segment of one animal's cortical rest data). Both examples

demonstrate an important property of crackling noise: rapid swings in fluctuations with no obvious periodicity, and little obvious tendency to remain around certain values. Using the same animal's data as in 1A, we can demonstrate what avalanches look like inside a raster of all binned cortical spiking data (Fig. 1B), where the raster represents just another way to see what comprises the crackling noise of spiking in detail. Here we can expand two smaller avalanches (orange highlight for Fig. 1C & blue highlight for Fig. 1D). As in the methods, each of the avalanches are bounded by 5 ms frames or bins of inactivity across the whole array, and, consistent with other groups, even these small avalanches have a characteristic inverted-U shape (Friedman et al., 2012). As can be expected, these are a miniscule fraction of avalanche shapes, lengths, and magnitudes, but as can be seen, they follow a general pattern.

Demonstration of log-log fits and further scale-free properties of in vivo avalanche data

All resting-state avalanches in each region had a duration (number of 5 ms bins) and event power or magnitude (spikes per bin) assigned, and subsequently these were plotted using a custom histogram and logfit function; Fig. 2A shows representative resting state cortical data, while 2B shows corresponding striatal data, where each data point represents an automatically binned group of avalanche sizes and each bin holds 10 spikes. As indicated in both panels, the goodness of log-log fit R^2 was above 0.85 in both the cortex and striatum, with a slope (α) near-3/2, close to the golden ratio, theoretical calculations, and experimental (neurobiological) observations of this value (Zapperi et al., 1995; Friedman et al., 2012; Timme et al., 2016; Cocchi et al., 2017). When we compared dug and control α values for resting log-log plots, they did not differ in a statistically significant way for the cortex (Fig. 2C; Mann–Whitney U test, $p = 0.578$) or striatum (Fig. 2D; Mann–Whitney U test, $p = 0.643$). Considering that these data all fall within physiological parameters, we were confident that our own toolbox can reliably extract avalanche data from our datasets. Although we didn't find differences in log-log fit exponents

between drug groups, we are aware that systems operating at criticality have more features such as universality in their data, and many systems that do not operate at criticality do exhibit α values similar to critical data, demonstrating that fit exponents of power laws are not sufficient to demonstrate criticality (Friedman et al., 2012), and *a priori* may not provide enough information for quantitative tests for a meaningful result with observed data. However, these results do point to our data having scale-free properties, as the slope of the data distribution is same across all scales, following a power law, suggesting that our data are fractal.

Avalanche shape collapses and shape error presentation

As showing fit exponents doesn't prove our recorded units are operating in a scale-free, critical network, we set out to develop a toolbox to take all avalanches at any time point, for example, at rest or after cue presentations, and collect those that had the same length or duration and collapse them to a normalized duration and event power (z-score of spikes per time point). Our reasoning was that if our data were fractal, self-similar, or critically tuned, then universally, at any duration, avalanches should look the same. Shapes that look less similar, either through having greater deviation or error from a mean shape amongst all same-length collapses or a smaller pairwise correlation coefficient between same-length pairs would then be said to less critically tuned or less fractal. Figure 3A demonstrates what six mean avalanche shapes of different durations appear like when plotted with their standard error at each frame. We then normalize each mean shape by using a z-score (Fig. 3B, left) and subsequently normalize the duration of each shape (Fig. 3B, middle). One animal's entire avalanche criticality or universality profile in one timeseries, such as at rest, can be visualized by then taking the mean of all interpolated mean shapes and plotting the standard error (Fig. 3B, right). Each animal's dataset can be treated similarly, and one can roughly see a qualitative difference in universality profiles in drug-treated mice (Fig. 3C, left) and control mice (Fig. 3C, right). Since all

data are treated the same and have a set of same-length interpolated collapses that represent mean avalanche shapes of originally different durations, we applied two different quantitative tests on these data to ascertain differences between resting state critical tuning in drug-treated and control mice in cortex and striatum.

Cocaine enhances critical tuning and correlates to conditioned pupillary response in cortex

We used an established formula, the median absolute error (MAE), as the first quantitative test to see how critically tuned each brain region in each animal at rest was. This formula is one of a number of summary statistics that are used to measure data dispersion, fitting for when one measure's how close data points are to each other (Gauss, 1816; Walker, 1929). The MAE can be written as (1)

$$\frac{1}{n}\left(\sum_{1}^{100} median(\ |\ [(\forall n)z_i\ ]\ -\ \tilde{z}_i\ |\ )\right) \qquad (1)$$

Where n is the number of collapse shapes in that condition, $[(\forall n)z_i]$ refers to a matrix of all n z-scores, $\tilde{z}$ is the median z-score, and i is the time within the nth normalized, interpolated collapse shape, which ranges from 1-100. Since this is a unitless number representing absolute error, the smaller it gets for each animal in each condition, the more similar each median collapse shape is to each other, and thus, more self-similar or fractal. Using this MAE metric, we found that in the cortex, drugged mice were more tuned to criticality than controls (Fig. 4A; Mann–Whitney U test, p = 0.042); this did not hold true in the striatum (Fig. 4B; Mann–Whitney U test, p = 0.158). This is intrinsically not intuitive, as one familiar with addiction research and cognitive bias towards addicts (Ahern et al., 2007) would expect hypoactivity and less critical or less computationally beneficial brain states in drug-treated brains. However, this is in line with our previous observations on slight resting state cortical hyperactivity.

Given that a critical brain state is thought to prime the brain towards appropriate stimulus response, we correlated our previous pupillary dilation results with our MAE data per region. We found that resting cortical MAE universality in the drug-treated group had a statistically significant correlation to pupillary dilation to both cues (Fig.5A; drug cue r_s = -0.802, p = 0.007, saline cue r_s = -0.924, p = 0.0003). This is surprising, as we previously only observed cortical and striatal inhibition proportional to pupillary dilation to the drug-paired cue, not both cues. Here, neither the drug-group striatal recordings (Fig.5B; drug cue r_s = 0.200, p = 0.584, saline cue r_s = 0.067, p = 0.865), nor the control group cortical (Fig.5C; saline cue 1 r_s = -0.643, p = 0.139, saline cue 2 r_s = -0.054, p = 0.883) and striatal recordings (Fig.5D; saline cue 1 r_s = -0.179, p = 0.713, saline cue 2 r_s = 0.541, p = 0.221) had statistically significant correlations of MAE to pupillary dilation.

To answer a potential objection to our usage of a proxy for how closely data collapses resemble each other, we took our resting state collapses in the cortex and striatum for each animal and performed pairwise correlations of each interpolated collapse, such that low duration collapses would be compared to longer and longer collapses, and vice versa. The resulting data are sets of correlation matrices based on pairwise collapse timeseries of control data (Fig. 6A; only cortical data shown, Pearson's r shown in top right, p-values of each pairwise comparison shown in bottom left) and drug-group data data (Fig. 6B; only cortical data shown, Pearson's r shown in top right, p-values of each pairwise comparison shown in bottom left). Essentially, this is mathematically one of the simplest ways to demonstrate similarity in timeseries data, but comes with the caveat of interpreting the deluge of correlation coefficients.

We first demonstrated that there is a strong correlation between number of collapse types at rest, that is, different shapes or durations of avalanches, and pupillary dilation only in the cortical drug data (Fig.7A; drug cue r_s = 0.936 p = 0.0002, saline cue r_s = 0.888, p = 0.0012), representing a close similarity to how MAE correlates to dilation and arousal. This of course

makes sense, given that if a system is operating closer to criticality, more various data points across different timescales would be observed; this takes the form of more durations of avalanches to observe. As before, neither the drug-group striatal recordings (Fig.7B; drug cue r_s = -0.152, p = 0.682, saline cue r_s = 0.006, p > 0.999), nor the control group cortical (Fig.7C; saline cue 1 r_s = 0.679, p = 0.110, saline cue 2 r_s = 0.234, p = 0.620) and striatal recordings (Fig.7D; saline cue 1 r_s = 0.487, p = 0.271, saline cue 2 r_s = -0.509, p = 0.233) had statistically significant correlations of collapse types to pupillary dilation.

Subsequently, we compared counts of all statistically significant ($p < 0.05$) positive Pearson's r values in the drug treated vs. control condition, and in the cortex found a statistically significant increase in positive correlation count for drug-treated mice (Fig. 7E; Mann–Whitney U test, p = 0.043). Again, this could not be found in the striatum (Fig. 7F; Mann–Whitney U test, p = 0.471). These data are consistent with our previous MAE result, showing that even when we directly compare collapse shapes to each other, we do find more often that collapse shape timeseries data are correlated, and thus self-similar and closer to being fractal in the drug-treated condition. Interestingly, this finding also is only in the cortex and not in the striatum, suggesting that if any malleability in the system occurs early in transition to addiction, the first place it could manifest is the cortex.

Cocaine increases complexity of crackling noise in the cortex while decoupling from criticality

As outlined above, criticality and information entropy and system complexity are related. By comparing total individual neural entropies to the joint entropy of the system, the degree of coordination, dubbed integration of neural firing can be measured (Tononi et al., 1994; Timme et al., 2016), highly similar to the dynamic correlation or "total correlation". This property is thought to confer systems with better information storage and ability to have sweeping responses to even slight inputs. We used a publicly available complexity toolbox (Marshall et al., 2016) on our

own resting state firing rasters (the underpinnings of neural crackling noise) to see how cocaine treatment alters quiescent complexity, with the hypothesis that complexity would be enhanced, as was MAE and counts of statistically significant positive pairwise correlations of collapses.

Complexity represented by integration is naturally a difficult metric to interpret, so using the aforementioned toolbox, we demonstrate the complexity of a small chain model of a simple system of 12 pseudo-neurons with three model constraints: Random spiking (Fig. 8A, top), complex spiking (Fig 8A, middle), and ordered spiking (Fig. 8A, bottom). The resulting integration of the random data reveals no integration or coordination, similar to white-noise of the Ising model when temperatures are very high (Fig. 8B, top), while the ordered state, similar to the low temperature Ising model, has a perfectly linear integration curve, indicating that although there are interactions between pairs of neurons, there is no variability in them. Only the complex data have a nonlinear integration, suggesting variable coordination, and thus, a non-zero complexity.

Using insight gained from this example, we used our *in vivo* resting state crackling noise data in the complexity toolbox and once again compared drugged mice to control mice in both brain regions. As predicted, we found that drugged mice have enhanced complexity in the cortex, compared to control mice (Fig. 8C; Mann–Whitney U test, $p = 0.025$), but not in the striatum (Fig. 8D; Mann–Whitney U test, $p = 0.458$). However, we still wanted to ascertain if there was a direct correlation between our MAE universality score and complexity, as they are related but not necessarily the same thing. We found, as expected, a strong correlation between MAE universality and complexity in our resting state data in the control mice in the cortex (Fig. 9A; $r_s = -0.857$, $p = 0.024$) and striatum (Fig. 9B; $r_s = -0.786$, $p = 0.048$). Surprisingly, correlation was broken in drug-treated mice in both the cortex (Fig. 9C; $r_s = -0.596$, $p = 0.071$) and striatum (Fig. 9D; $r_s = -0006$, $p > 0.999$). As this is the only result seen that runs counter to expectations

about drugs and criticality, it could be that this is one of the features that dictates aberrant drug-induced plasticity at the systems level.

Discussion

In pursuing a basis for observing no cortical hypoactivity previously, we've developed our own novel methods to detect neural avalanches that solve an ongoing issue in the field of neural criticality by quantifying universality and critical tuning (Marshall et al., 2016; Shaukat and Thivierge, 2016). We've also observed criticality in deep brain structures such as the striatum and medial prefrontal cortex *in vivo*, as well as documented effects of a potent narcotic on neural criticality, both representing pioneering advances in systems-level addiction research and SOC research.

The significance of obtaining crackling noise data and avalanches therein from rasters of neural firing *in vivo* from the mPFC and striatum should not go unnoticed. This represents the first usage of nanofabricated multishank electrode recordings to approach deep-brain criticality analysis *in vivo* in awake, behaving mice. Typically, recordings for avalanche measurements are made from organotypic cortical slices with large inter-electrode spacing of 200 μm or more (Beggs and Plenz, 2004; Timme et al., 2016). Even the most advanced *in vivo* murine recordings used in avalanche analysis only had 32-channels with a 200 μm inter-electrode spacing and 400 μm inter-shank distance(Gautam et al., 2015); even then the recorded sites were in superficial barrel cortex in anesthetized subjects. Compared to other *in vivo* experiments, our inter-electrode spacing is almost an order of magnitude closer, we used more than an order of magnitude more electrodes, and we had shanks half the distance apart, representing a large difference in recording capabilities. Despite these distinctions, we were still able to extract avalanches using criteria by other groups, lending credence to the concept of SOC as a ubiquitous feature of neural systems.

When plotting avalanche size in log-log coordinates, we were able to replicate power law fits as other groups have, with similar α fit exponents near the golden ratio (Cocchi et al., 2017). Interestingly, despite having dosed mice with cocaine, their power law fit exponents didn't change compared to control mice, which might due to the differences in recording technology as outlined above, or in how we designated our inter-avalanche interval of 5 ms. For example, other groups have used an interval dependent on the subjects own neural activity levels (Priesemann et al., 2013) which may skew avalanches towards a more accurate level. Additionally, as we did not compare our fit to multiple other potential model fits, such as doubly truncated power laws or exponentials, or use an advanced logfitting tool specifically optimized to our datasets, our fit and subsequent fit exponents may deviate from what other groups might obtain using more advanced mathematics. On the other hand, the fact that we were able to use a stock log-log fit toolkit and apply it to obtain fits with $R^2 > 0.85$ implies that these were successful. Ultimately, the power law aspect of our dataset is only one facet of the neural criticality hypothesis, and whether they vary due to drug treatment does not necessarily overturn the results on universality that we examined. As it currently stands, few studies on drug-based critical tuning exist, and have only worked with bath applications of channel blockers in organotypic slices (Shew et al., 2009). It would make sense that α would be altered in these experiments, and in light of these studies, our negative result is less surprising, given that incubation of craving is much more physiologically subtle.

When we collapsed our datasets, we consciously chose to avoid using methods difficult to understand or replicate mathematically by normalizing event power or spikes per frame using a z-score and normalizing collapse duration. Although there may be merits to choosing scaling parameters to collapse avalanche shapes (Friedman et al., 2012; Priesemann et al., 2013), our opinion is that a true test of universality is to automatically scale each collapse and judge their shapes based on errors and timeseries correlations, after all, scale should not matter if

universality means that the fractal shape is the same no matter the scale. Considering this, we believe our method to be a most parsimonious method in neural criticality research, as rather than spend effort on making each avalanche collapse uniform to an arbitrary standard, we fit them to a normalized standard and evaluate purely on shape.

Our evaluation of interpolated mean avalanche collapses represents marked progress in the SOC field, whereas before, other groups have not been able to quantify the universality or universal scaling aspect of neural criticality (Marshall et al., 2016). At best, use of shape modeling, generalized linear modeling, or shape fitting transform data to generate testable variables. At their worst, transforms overly generalize data and enable tests on data separate from the real collected values and are unintelligible (Shaukat and Thivierge, 2016). As we have already attempted to perform polynomial fitting on collapses (data not shown) with fit coefficients as test statistics, we understand that although appealing, ultimately, fits are uninformative, as they skew the issue of testing universality into a test of whether an arbitrarily chosen fit coefficient makes a collapse appear to be similar to an inverted parabola. The most important aspect of using the MAE or quantitatively assessing pairwise correlation matrices of collapses is that the data are not transformed beyond normalizing, and both directly quantify dissimilarity or similarity (respectively). The metrics we used are well understood, computationally simple, and open up data to further easily interpretable analyses as with correlations.

That we observe a resting state or quiescent enhancement in criticality through use of two mathematically different metrics, MAE and significant positive pairwise correlation counts only in the drug-treated mice, only in their cortex, makes mathematical sense and further legitimizes our findings. We acknowledge that it doesn't make sense at first glance, given the abundance of data supporting cortical hypoactivity (Jasinska et al., 2015) and that the stereotype of an addict is one who is aimless, lacking in moral character and self control, or in the throes of withdrawal, unable to think clearly. We offer a parsimonious explanation for this

result in that only during the transition to addiction, there is an uptick in resting firing that we've observed and now expanded upon. Following this, hypoactivity sets in, again, only in the cortex, and in the literature there exists support for this scenario in recently abstinent subjects (Mayer et al., 2013). Indeed, our current study links the hyperactivation to a marker for clinical severity of addiction (pupillary dilation) and a potential marker for clinical outcome (critical tuning), a direct answer to Mayer et al. That the striatum's critical tuning is unaffected is less of a surprise, given that we previously saw no resting-state changes in it. Maladaptive plasticity within the striatum during incubation is thought to take place over multiple weeks (Ma et al., 2014), and thus how incubating craving affects criticality remains an open question.

Even more curious, however, is that both the pupil dilation elicited by the drug-paired and saline-paired cues was highly anticorrelated to resting state MAE universality in the cortex of drugged mice and highly correlated to number of collapse types. We assumed initially that the dilation due to the drug paired cue would correlate inversely to MAE universality and correlate to shape count for two reasons. For one, previously, we saw frontostriatal inhibition correlated to dilation only to the drug cue, so assuming only one cue would have an impact would make sense. Secondly, salient environmental stimuli are reported to push the brain away from peak quiescent criticality (Cocchi et al., 2017) and thus animals having greater arousal and pupillary dilation would have likely had cue-related activity that balances out whatever critical tuning exists at rest. However, we hadn't anticipated that the dilation due to both cues would correlate to our resting criticality metrics. Our interpretation is twofold: The degree of critical tuning at rest in the cortex corresponds to how aroused they become by drug paired cues, and given that both cues share a sensory modality, they must be discriminated. This discrimination, likely the variable inhibition we previously found, occurs not during resting firing, but during and after the cue to encode a proper response, and resting critical dynamics subserve such a response. We posit that the potential to respond to the appropriate cue is built into the resting representations

of both cues, and when the appropriate cue arrives, metabolic resources are spent on cortical inhibition when the more salient cue arrives. In the controls, since no cues signify drugs or a cue that is particularly beneficial to the subjective experience, the correlations don't exist.

In line with our observation about criticality being enhanced at rest in the cortex after brief cocaine abstinence, a measure of complexity was also enhanced in the same group of mice. We find that, as recently hypothesized (Timme et al., 2016), degree of critical tuning and complexity are highly correlated in both the cortex and striatum of our controls. Of note, however, is that this correlation breaks down in both regions in the cocaine-dosed mice. The aberrant loss of a link between complexity and criticality could be a system-state change that denotes a transition to addiction, much like how we observed a spontaneous increase in corticostriatal LFP between 25-45 Hz. It should not be ignored that is was the only significant multi-region result from these sets of analyses, suggesting a non-trivial link to our previous report on inhibition and LFPs.

Ultimately, these results represent a great step closer in understanding variability in how both addiction and neural criticality manifest *in vivo.* At its core, this work enhances the criticality hypothesis in neural systems with high-density, awake behaving *in vivo* data. We've provided solid evidence for early stage resting cortical hyperactivity after cocaine doses, rather than hypoactivity, as well as multiple quantitative metrics to address avalanche collapse shape universality, a more thorough way to assess critical dynamics and fractal physiology, rather than relying on power laws only.

One potential translational and therapeutic application of this work is to assess critical brain dynamics as a quantitative marker of addiction state and progression, using EEG (Irrmischer et al., 2018). Mindfulness-based cognitive therapy or meditation can already be used to great effect in handing depression, PTSD, and other brain disorders (Hayes et al., 2011). Adding real-time, non-invasive EEG feedback on the subject's attention, arousal, and critical

dynamics, rather than a generalized “one-size-fits-all” approach to therapy, could aid in controlling the progression of addiction, or other disorders, as well as enhance the quality of life for practitioners thereof. Even advances in engineering a true artificial intelligence (Avizienis et al., 2012; Sillin et al., 2013) could be made by further understanding of how brain states utilize SOC to function. It is our hope that by establishing our recording paradigm *in vivo*, further advancements in understanding basic cognition through avalanche analysis will be made.

Figures

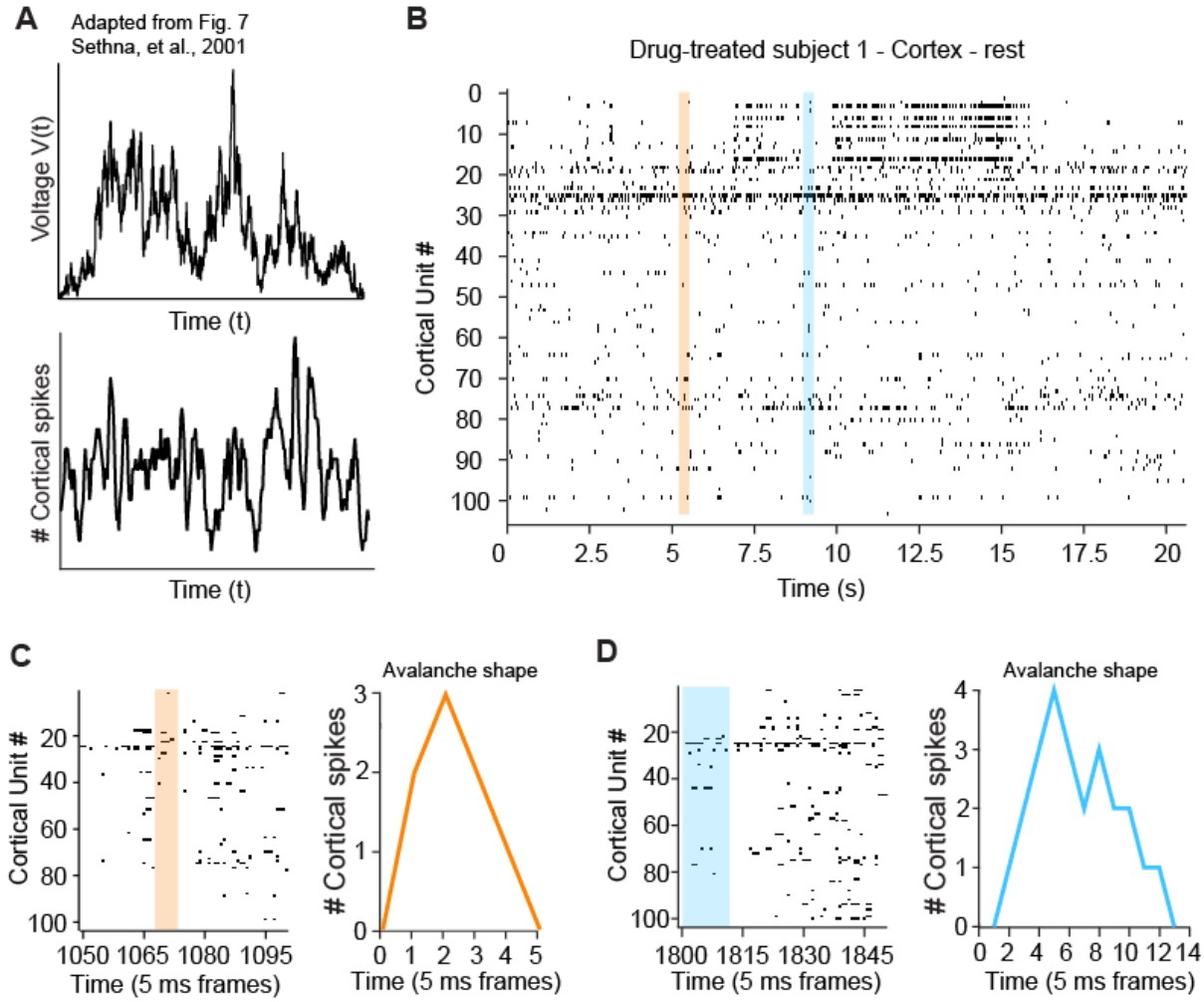


Figure 1. Demonstration of scale-free crackling noise and avalanche shapes in vivo. **A**, Example of crackling noise (Barkhausen noise) from a voltage pulse (number of domains flipped per unit time) during a single large avalanche in a magnet (top, adapted from Sethna et al., 2001) and crackling noise from the cortex of one mouse due to neurons firing during rest. Generally speaking, crackling noise manifests similarly, irrespective of scale. **B**, A segment of the firing raster that comprises the crackling noise in **A**. **C**, Expanded raster of the orange trace in **B** (left) and the corresponding avalanche shape (right). **D**, Expanded raster of the blue trace in **B** (left) and the corresponding avalanche shape (right).

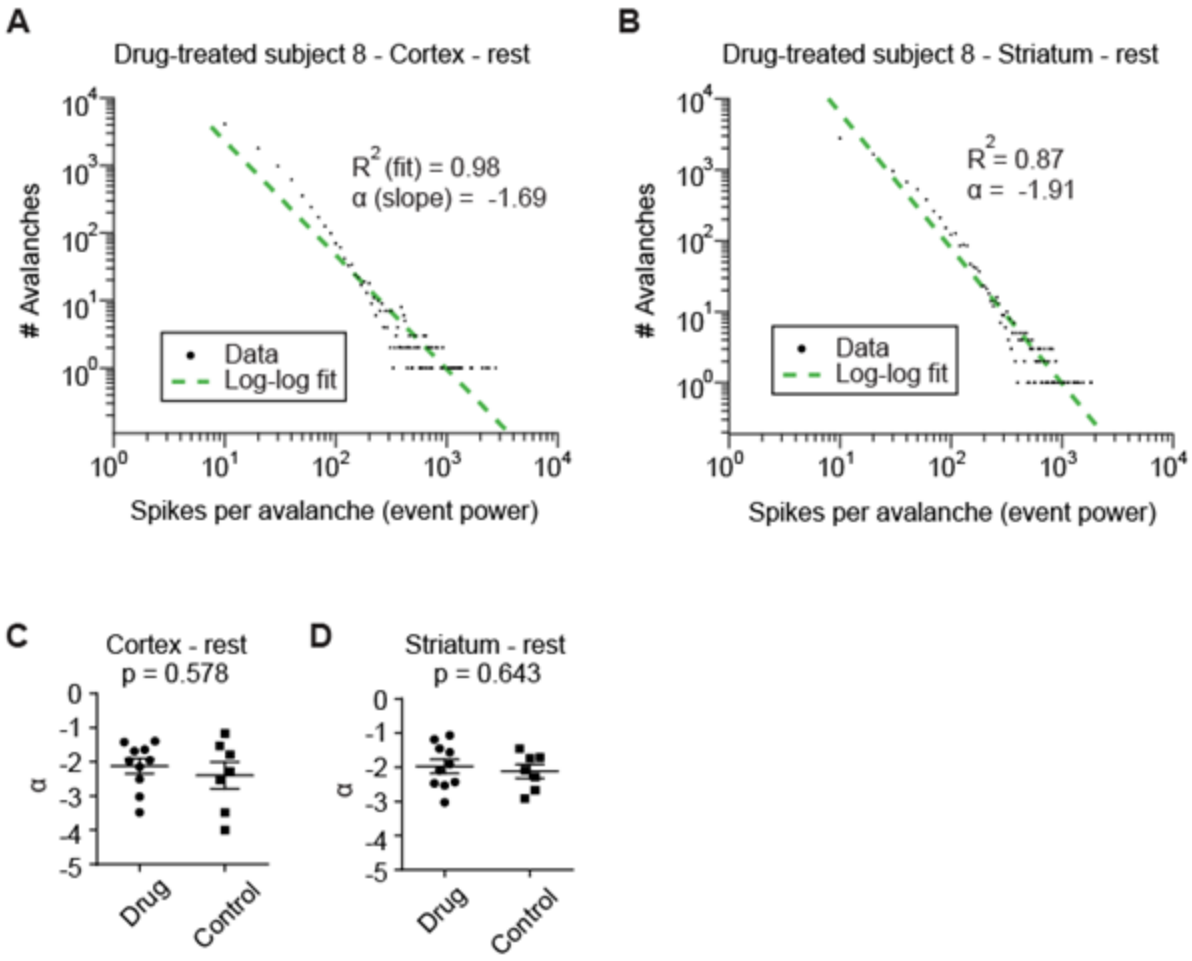


Figure 2. Demonstration of log-log fits and further scale-free properties of in vivo avalanche data. **A**, Cortical avalanches plotted with event power vs. avalanche count in log-log coordinates follow a power law with R^2 = 0.98 and α (slope) = -1.69. Striatal avalanches from the same subject and plotted in the same way as in **A**, with R^2 = 0.87 and α (slope) = -1.91; both sets of values have high goodness-of-fit and α close to a negative golden ratio (-3/2), consistent with other scale-free, critical phenomena in nature and in organotypic slices. **C**, Resting α did not differ between drug and control groups in the cortex (Mann–Whitney U test, p = 0.578). **D**, Resting α did not differ between drug and control groups in the striatum (Mann–Whitney U test, p = 0.643).

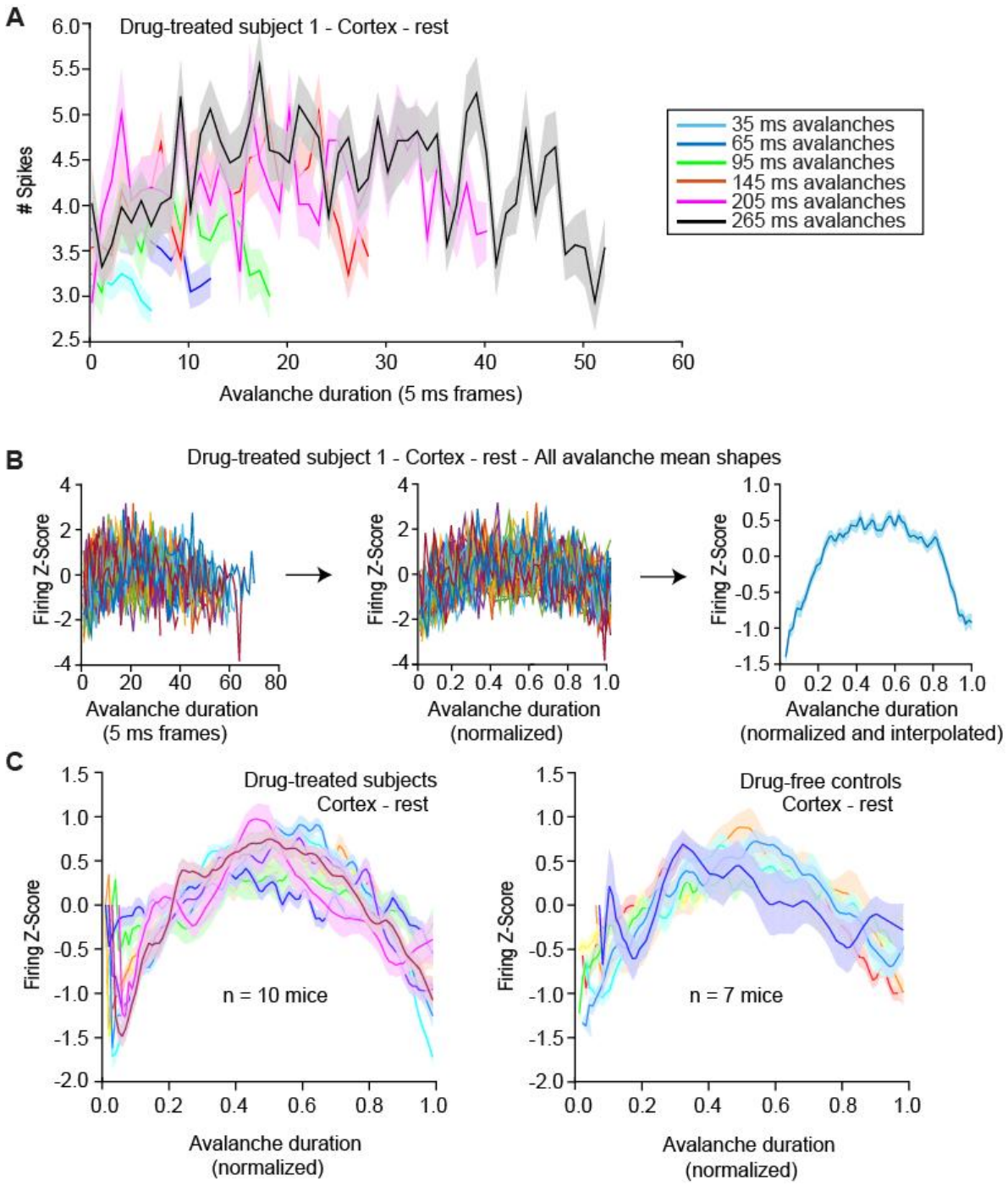


Figure 3. Avalanche shape collapses and shape error presentation. **A**, Six mean avalanche shapes are plotted (shaded areas: SEM), which comprise all avalanches that shared the indicated duration. Generally speaking, both long and short avalanches appear to have similar shapes at differing scales. **B**, The collapse process starts by taking all mean avalanche shapes and normalizing their firing per frame (left), then the total duration (middle). At this point, each mean shape is collapsed and plotted over each other. To obtain a subject's universality profile, we interpolate each shape to have 100 timepoints and take the mean of that shape (right) **C**, Shape universality profile for cortex in drug-treated mice (left) and control mice (right).

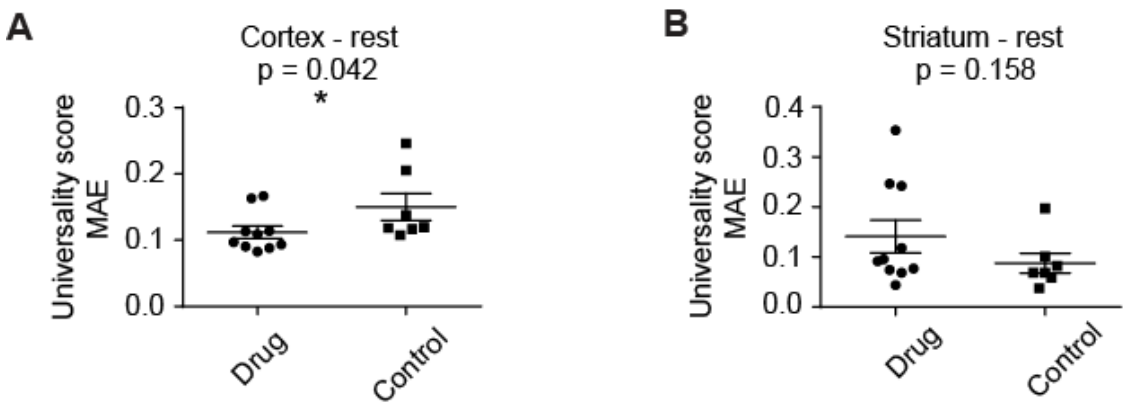


Figure 4. Cocaine enhances critical tuning in the cortex of drug treated mice. **A**, Cortical data at rest have a lower MAE and are more tuned towards criticality in the cocaine-dosed mice (Mann–Whitney U test, p = 0.042). **B**, Striatal data at rest in drug-treated and control mice appear to be equally tuned towards criticality (Mann–Whitney U test, p = 0.158).

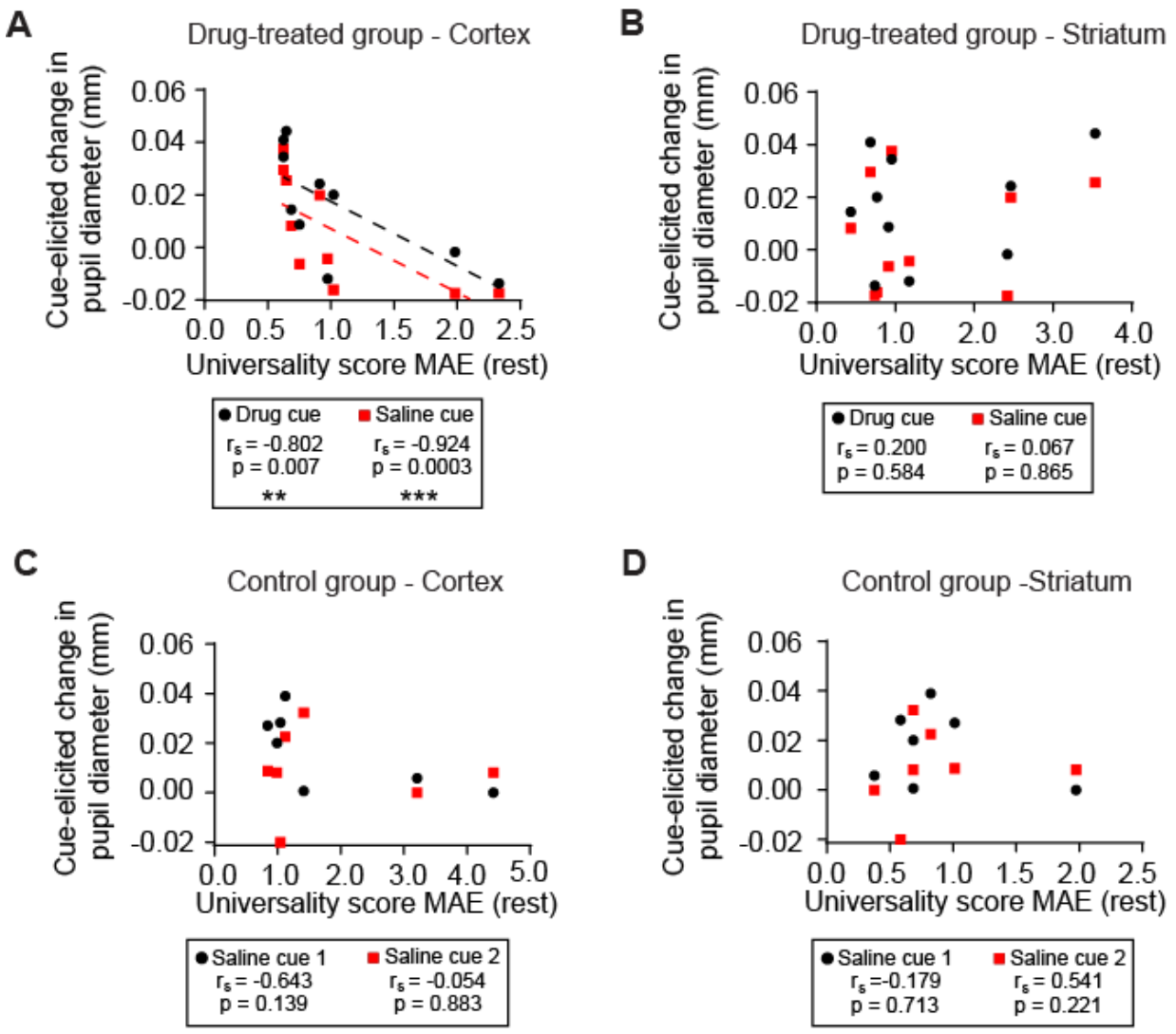


Figure 5. Cocaine treatment makes critical tuning inversely correlate to both conditioned pupillary responses in cortex. **A**, Resting MAE in the cortex of drug-treated mice inversely correlated to both drug cue- elicited pupil change diameter (r_s = -0.802, p = 0.007) and saline cue-elicited change in pupil diameter (r_s = -0.924, p = 0.0003), indicating heightened arousal is correlated to resting criticality. **B**, Resting MAE in the striatum of drug-treated mice did not correlate to drug cue (r_s = 0.200, p = 0.584) or saline cue (r_s = 0.067, p = 0.865) elicited change in pupil diameter. **C**, Resting MAE in the cortex of control mice did not correlate to the first saline cue (r_s = -0.643, p = 0.139) or second saline cue (r_s = -0.054, p = 0.883) elicited change in pupil diameter. **D**, Resting MAE in the striatum of control mice did not correlate to the first saline cue (r_s = -0.179, p = 0.713) or second saline cue (r_s = 0.541, p = 0.221) elicited change in pupil diameter.

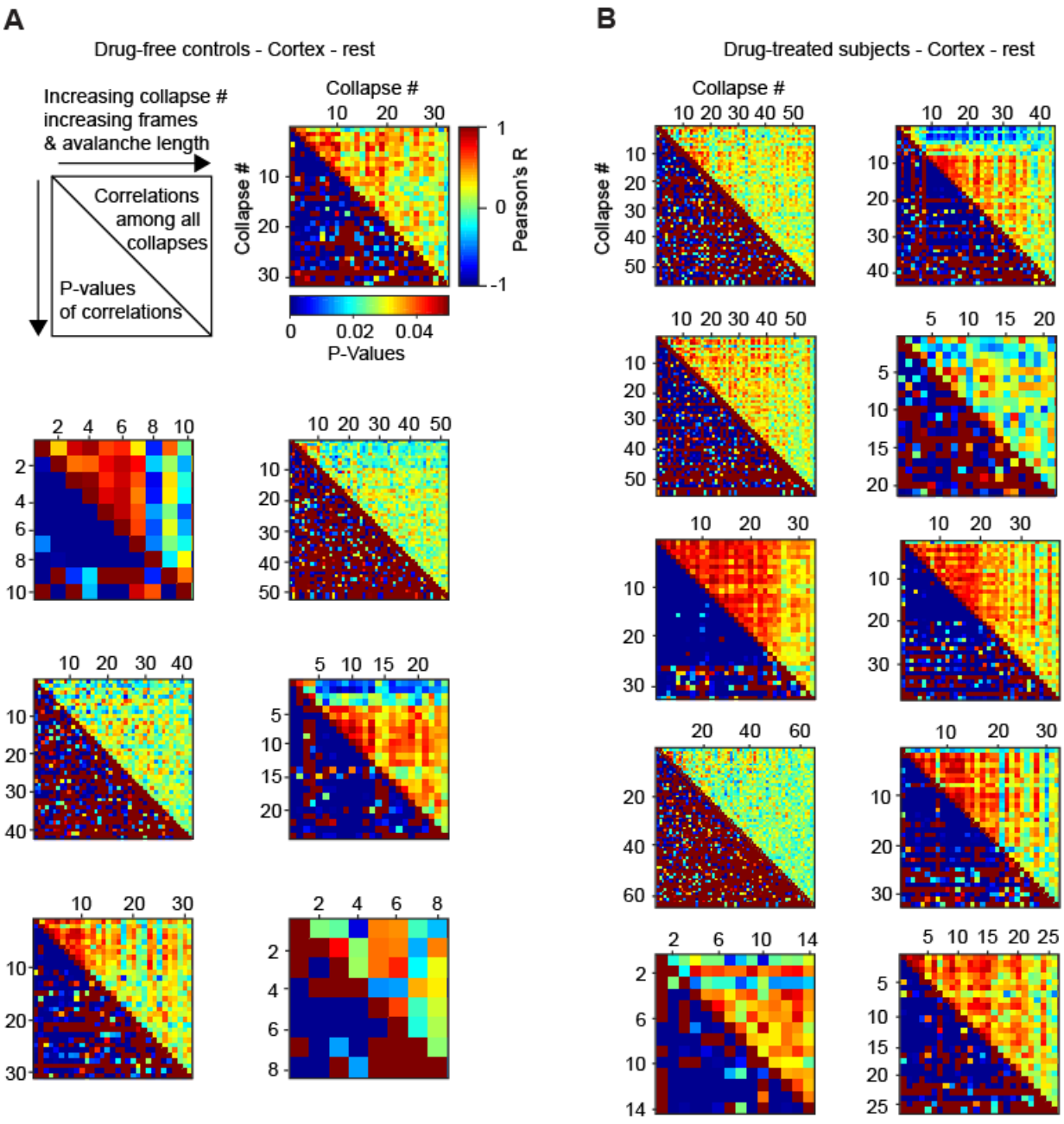


Figure 6. Demonstration of correlation matrices of mean interpolated collapses. **A**, All control subject data plotted for cortical collapse correlations and a legend for interpreting each dataset (top left). Note that the cutoff for significance is p = 0.05, and in most cases, p-vales were very low (p < 0.01) or above 0.05. Interpolated collapse data are amenable to correlation-based analyses, as they are essentially normalized timeseries plots of neural firing. **B**, Each drug-treated subject's cortical collapse correlation matrices with the same legend as in **A**.

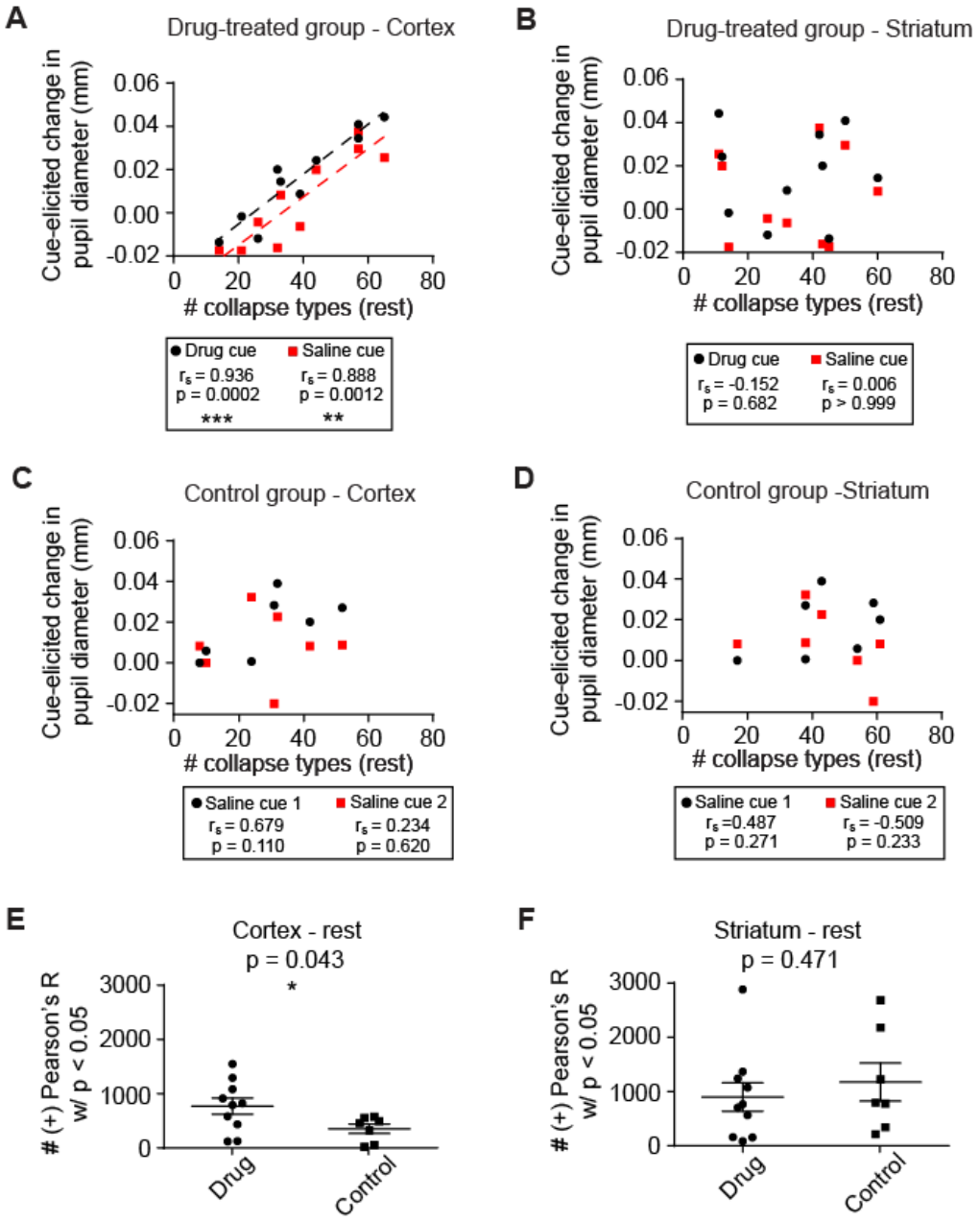


Figure 7. Correlation analysis shows cocaine makes critical tuning correlate to both conditioned responses in cortex and enhances critical tuning at rest. **A**, In the cortex of drug-treated mice, number of collapse types (different durations) correlates to drug cue (r_s = 0.936 p = 0.0002) and saline cue (r_s = 0.888, p = 0.0012) elicited change in pupil diameter. **B**, In the striatum of drug-treated mice, number of collapse types did not correlate to drug cue (r_s = -0.152, p = 0.682) or saline cue (r_s = 0.006, p > 0.999) elicited change in pupil diameter. **C**, In the cortex of control mice, number of collapse types did not correlate to the first (r_s = 0.679, p = 0.110) or second (r_s = 0.234, p = 0.620) saline cue-elicited change in pupil diameter. **D**, In the striatum of control mice, number of collapse types did not correlate to the first (r_s = 0.487, p = 0.271) or second (r_s = -0.509, p = 0.233) saline cue-elicited change in pupil diameter. **E**, Cortical data at rest have a higher count of statistically significant positive correlations in the cocaine-dosed mice (Mann–Whitney U test, p = 0.043). **F**, Striatal data at rest in drug-treated and control mice show no such difference in statistically significant positive correlation counts (Mann–Whitney U test, p = 0.471).

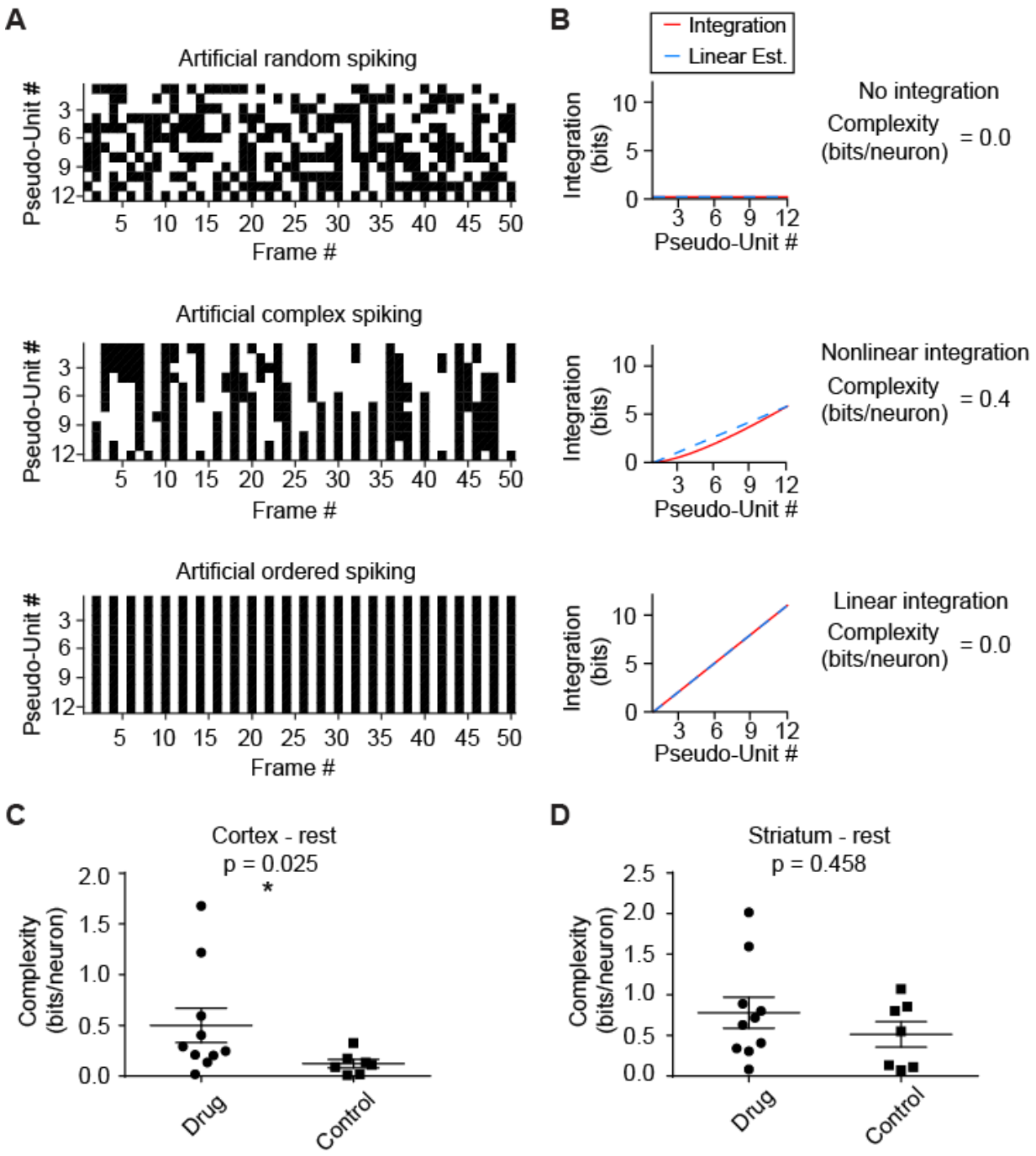


Figure 8. Cocaine increases complexity of crackling noise in the cortex. **A**, Pseudodata spike rasters under three different imposed regimes: Random uniform (top), complex (middle), and ordered bottom). These illustrate what we refer to when speaking of criticality. **B**, Integration plots and complexity values for each plot based on the pseudodata in **A**. Only the complex data has a nonlinear, scale-variant integration, while random data has no integration, and ordered data has perfectly predictable integration. Due to this, only complex data has a nonzero complexity. **C**, Cortical data at rest have a higher complexity score in the cocaine-dosed mice (Mann–Whitney U test, p = 0.025). **D**, Striatal data at rest in drug-treated and control mice show no statistically significant difference in complexity (Mann–Whitney U test, p = 0.458).

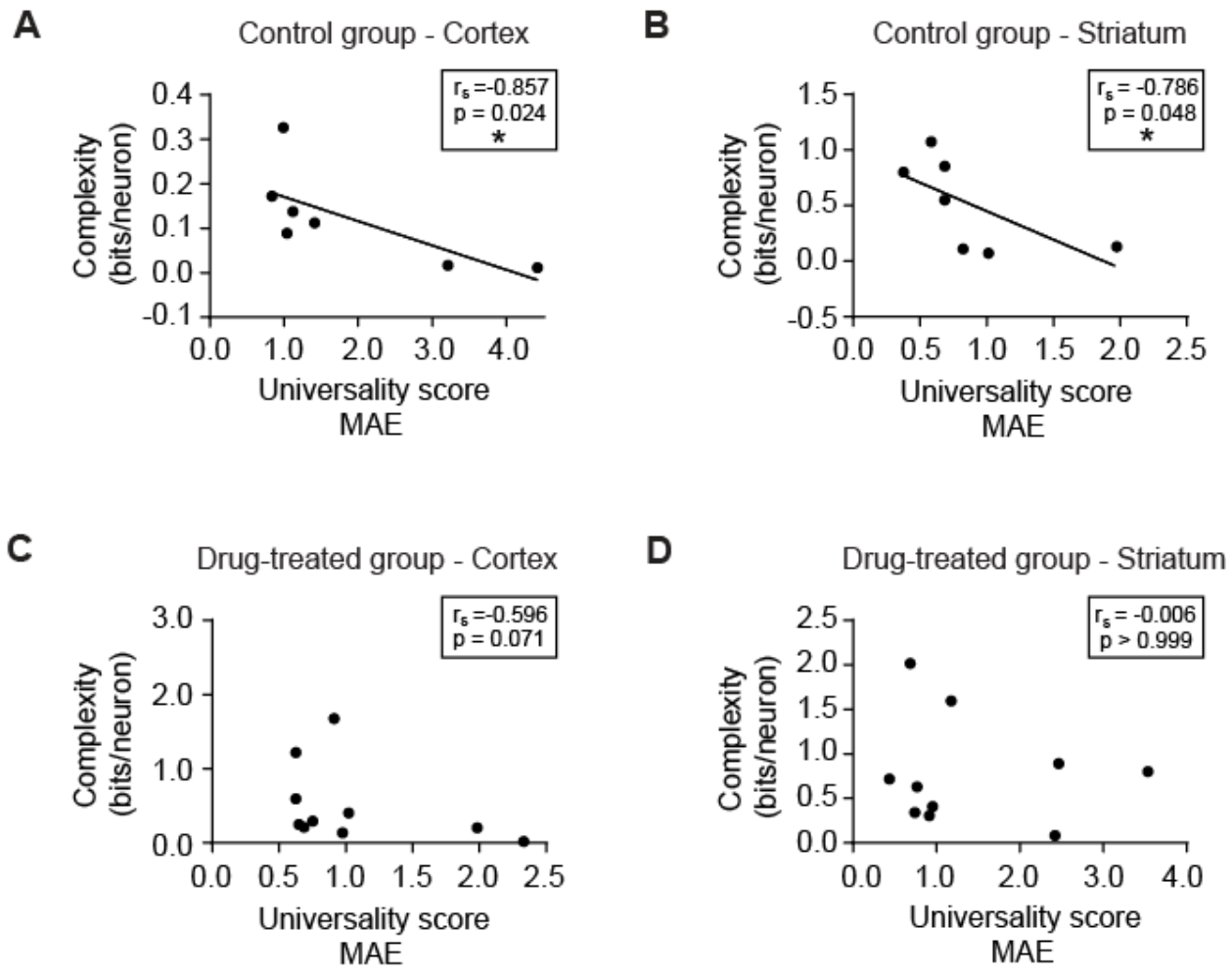


Figure 9. Cocaine decouples critical tuning from complexity in cortex and striatum. **A**, In control mice, MAE was inversely correlated to complexity score in the cortex (r_s = -0.857, p = 0.024), which we infer to mean that as data have more critical tuning with a lower MAE, they increase complexity, consistent with recent work. B, As in the cortex, striatal MAE scores also inversely correlated with striatal complexity scores (r_s = -0.786, p = 0.048). **C**, No statistically significant correlation between MAE and complexity score could be found in the cortex of drug-treated mice (r_s = -0.596, p = 0.071). **D**, No statistically significant correlation between MAE and complexity score could be found in the striatum of drug-treated mice (r_s = -0006, p > 0.999).

GENERAL DISCUSSION

This thesis provides evidence that cocaine can drive autonomic conditioned responses, the variability of which correlate to corticostriatal inhibition and critical brain dynamics. In order to reach that level of analyses, we had to first demonstrate that mice could even perform a CR task with readily interpretable data after drug usage while awake on a moving ball, and refine our recording and behavior procedures, given all the degrees of freedom our task entailed. We feel that the contributions we've made to addiction research will identify modes of behavioral therapy that rely on measures of cortical SOC tuning, and aid in the design of tailor-made therapies depending on the stage of addiction each patient is at. What we've shown for brains operating at different levels of criticality will help further cement the neural criticality hypothesis and enable other questions about neural network dynamics, in order to understand the fundamentals of cognition.

We first wanted to study systems-level correlates of addiction with our high-density probes in mice by means of hippocampal recordings in a VR environment, in order address what contextual stimuli encode that makes relapse so common. We piloted a study with head-fixed mice that received both odor and visual information in conjunction with noncontingent drug doses to test, but found that behavior responses were quite variable, despite successfully demonstrating classical psychomotor sensitization due to repeated cocaine injections. In this case, since mice either ran indiscriminately or successfully manifested a preference response about 50% of the time, we couldn't unambiguously state that the mean time spent in the drug-paired quadrant had changed compared to the pretest. This bimodal distribution of behavior had the potential to prohibit statistical analysis, so we changed the context to only include odor stimuli and reduce the need for mice to run to a virtual chamber; rather, we would have the readout become any movement in response to the drug-paired odor cue, as a type of autoshaped response.

Despite “bringing the cues to the mice” as opposed to CPP, which requires them to coordinate an exact response of moving and then stopping in a certain context, as well as subsequently suspending further movement, we failed to find any sort of statistically significant CR of appetitive locomotion, in both mice abstinent from cocaine for 24 hr and for 21 days. We did, however, find the same sort of bimodal distribution in behavioral responses, whereby half the mice hardly moved at all, and the other half ran quickly in response to the drug-paired cue. In those experiments, we recorded vast swaths of frontal cortex, including medial prefrontal and orbital cortex, as well as ventromedial to dorsolateral striatum. We failed to find population level indications of cue-responsiveness, and this may in part be due to the disparate responses of regions we recorded from, or from motion artifacts rendering us unable to infer cue-based responses vs. locomotor noise responses in cortex and striatum. To fix this, we subsequently decided to pare down the number of recorded regions, while keeping the number of channels the same with a redesign of the electrode array. To improve behavior as well as reduce the need for a locomotor response that would alter electrophysiology, we switched to performing pupillometry on mice, given that in the absence of any cues, mice tended to stay still on the ball. To overcome the appetitive motion found in half the subjects, we simply decided to not acclimate mice to the ball motion itself during the post-surgery habituation phase of the experiments, so motion overall would be unappealing to mice, thus ensuring pupillary responses would only come from the appetitive cues and arousal, not motion.

When we subsequently altered the experimental protocols, we did find a cue-based change in arousal in recently abstinent mice, where drug treated mice had a larger change in pupillary dilation to the cocaine-paired cue than the saline-paired cue, while no such change in cue value existed for saline cues in the control mice that never receive cocaine. Additionally, the cocaine-cue based change in dilation varied amongst all subjects, representing an approximation in how human substance abusers all respond to drugs differently or to different

degrees. Physiologically, we noticed a trend towards hyperactivity at rest, when we hypothesized that we would see hypoactivity in the cortex, as is the canonical observation. No population-based change in percentage of neurons encoding the cues existed for either group of mice in any brain region, but we could correlate the degree of inhibited neuronal firing in the cortex and striatum to pupillary dilation, suggesting a mechanism for cue-based arousal based on active inhibition. Not only that, but resting corticostriatal LFPs in the beta-gamma range were enhanced relative to controls, and also correlated to the subject's pupillary dilation. This suggests that aberrant corticostriatal connectivity exists in the resting condition and may be more apparent and played upon during cue-response relative to the degree at which the connectivity is changed, per-animal.

Since most experimenters observe hypoactivity in the cortex at rest and we saw slight hyperactivity and enhanced beta-gamma LFPs between the cortex and striatum, we decided to investigate further. We developed our own scripts to extract array-wide avalanches from spike rasters in our *in vivo* multisite data and compared these to other groups' data. Our results were very consistent with established neural slice data, suggesting our methods not only work, but also support the neural criticality hypothesis in both in deep cortex and striatum *in vivo* in awake animals, a novel result. We performed a mathematically simple and cogent method to collapse avalanches of different lengths, as data near a critical point are fractal and scale-free, and became the first to our knowledge to quantify how similar neural avalanche collapses are. We found that this universality was enhanced in drug-treated mice and correlated to both cues only in the cortex. System complexity, thought to be related to criticality, was only correlated to control data in both regions, suggesting that when drugs of abuse are taken consistently, the brain may be pushed into an aberrant critical state that lacks the concomitant information complexity that normally exists. That we found this result in controls is also significant, as it has only been previously hypothesized in neural data. These results support a model of cognition

that works at criticality, tuned to enhance complexity depending on the internal state of each individual, which can be usurped by drugs of abuse. This critical state, normally optimizing the brain to detect salient cues beneficial for survival, suddenly can be primed to detect drug-predictive cues. Further research is necessary to determine the time course of the onset of aberrant criticality, how reversible it is, whether a circuit-based mechanism exists to counteract it, and finally, if this mechanism is similar to other drugs of abuse like opioids and other brain disorders like depression or PTSD.

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