Review

Biological Psychiatry

Drug Cues, Conditioned Reinforcement, and Drug Seeking: The Sequelae of a Collaborative Venture With Athina Markou

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ABSTRACT

Athina Markou spent a research period in my laboratory, then in the Department of Anatomy in Cambridge University, in 1991 to help us establish a cocaine-seeking procedure. Thus we embarked on developing a second-order schedule of intravenous cocaine reinforcement to investigate the neural basis of the pronounced effects of cocaine-associated conditioned stimuli on cocaine seeking. This brief review summarizes the fundamental aspects of cocaine seeking measured using this approach and the importance of the methodology in enabling us to define the neural mechanisms and circuitry underlying conditioned reinforcement and cocaine, heroin, and alcohol seeking. The shift over time and experience of control over drug seeking from a limbic cortical-ventral striatal circuit underlying goal-directed drug seeking to a dorsal striatal system mediating habitual drug seeking are also summarized. The theoretical implications of these data are discussed, thereby revealing the ways in which the outcomes of a collaboration can endure.

Keywords: Alcohol, Cocaine, Conditioned reinforcement, Drug Seeking, Habits, Heroin

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Athina Markou was a highly motivated, energetic, and inspiring researcher with whom I not only collaborated, but also developed a close friendship. I am honored to have this opportunity to summarize what we did together in Cambridge and how this laid the foundations for advances made in the 25 years since she first spent time there. It was at the Society for Neuroscience meeting in St. Louis that I first met Athina, and she discussed with me her emerging interest in the mechanisms by which environmental stimuli become associated with the effects of addictive drugs to influence drug seeking and relapse. Her interest had been sparked by the seminal article by Gawin and Kleber (1) that established a link to what she had been studying in her outstanding Ph.D. research with George Koob, namely withdrawal mechanisms and the associated elevated reward thresholds in rats having self-administered cocaine for long periods. This aversive state was argued to model the anhedonia or dysphoria in cocaine-addicted humans in withdrawal that may drive persistent drug taking through negative reinforcement. Gawin and Kleber had described a recognizable withdrawal syndrome in cocaine addiction—something not acknowledged before 1980—that was not characterized by aversive physical symptoms, but instead by psychological symptoms including depression, anhedonia, anxiety, and fatigue (1). However, they also suggested that as these early withdrawal signs begin to dissipate, drug cues become progressively more important in eliciting craving and relapse the longer abstinence is maintained.

Two areas of research that might seem unrelated, but in fact were not, were being undertaken at the time in the Cambridge laboratory. In the first, following Taylor and Robbins's (2,3) demonstration that the potentiative effects of amphetamine on conditioned reinforcement depended on dopamine in the nucleus accumbens (NAcb), we went on to show that conditioned reinforcement itself depended on the basolateral amygdala (BLA) (4) and, later, its functional interaction with the NAcb core (NAcbC) (5). In the second, I had been investigating the neural mechanisms of sexual motivation and had developed a second-order schedule of sexual reinforcement in which male rats would seek access to a female rat in heat, and once having gained access would copulate to ejaculation (6,7). This enabled a measure of sexual incentive motivation to be obtained without relying on the measurement of performance, or consummatory, variables such as mounting and intromission. The prolonged period of instrumental responding for a female rat depended on the presentation of sex-associated conditioned reinforcers (8), and this also depended on the BLA and not on the preoptic area, which controls sexual performance in male rats (9).

The plan that Athina Markou and I discussed in St. Louis and then corresponded about was to establish a second-order schedule of cocaine reinforcement in rats so as to be able to measure 1) the motivation for cocaine without the confound of cocaine's effects on instrumental behavior and 2) the impact of drug cues established by pavlovian association between cocaine's effects and an otherwise neutral stimulus to support seeking behavior by acting as conditioned reinforcers (10). We had no track record in intravenous drug self-administration in Cambridge, so the stage was set

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for an ideal collaboration and a starting point from which we have never looked back.

Addictive drug-associated conditioned stimuli (CSs) can influence behavior in animals and humans in a number of ways. For example, they can elicit automatic approach behavior, thereby bringing the individual into the location of the CS where drug taking had occurred. This pavlovian approach behavior—or "sign tracking"—elicited by noncontingent presentation of drug cues to rats perhaps resembles the way that drug CSs are presented to addicted individuals in functional imaging experiments, although subjective states (e.g., craving), or sustained attention to and vigilance for drug cues, rather than behavioral responses, are more frequently measured in such studies (11–14). While sign tracking of alcohol cues has been demonstrated in rats, especially when the cue is located close to or at the site of alcohol delivery or the response location (15), there are still relatively few demonstrations of sign tracking to cues associated with intravenously self-administered drugs such as cocaine [reviewed in Saunders and Robinson (16)].

CSs can also potentiate instrumental seeking responses through a process now referred to as pavlovian-instrumental transfer (PIT), but long known previously as pavlovian (or conditioned) motivation. The great majority of PIT demonstrations have been in animals responding for ingestive rewards, including alcohol (17,18), but there are only isolated demonstrations of PIT in rats responding for intravenous cocaine, in which a modest effect was seen in highly specific circumstances that depended on prior bouts of CS and instrumental extinction (19). PIT has, however, been demonstrated in human subjects in response to a variety of drug and high-incentive food CSs with a shift from specific to general PIT in those subjects addicted to drugs (20). In a key demonstration, CS-potentiated smoking in humans was unaffected by satiety and therefore independent of the current incentive value of the drug (cigarette puffs; i.e., no specific PIT), and instead was expressed as a general motivational enhancement (i.e., a general PIT effect), providing evidence therefore of habitual drug use [(21), and discussed fully in Everitt and Robbins (22)]. The neural mechanisms underlying sign tracking and PIT are dissociable and involve the amygdala, NAcbC and NAcb shell, and their dopaminergic innervation, as well as related limbic cortical structures. This circuitry has been revealed primarily in studies of rodents responding for ingestive, rather than drug, rewards [and reviewed extensively elsewhere (16,23,24,25)].

However, it is the conditioned reinforcing properties of drug-associated CSs that exert the most powerful impact on drug seeking regardless of the procedure used to measure it. The important distinction between conditioned reinforcement and the processes (sign tracking and PIT) discussed above is that the CS is presented response contingently; it reinforces the instrumental response, acts as a subgoal of seeking behavior, and thereby enables an animal or human to tolerate and mediate delays to reinforcement (26). A cocaine-associated CS will in fact support the learning of a completely new instrumental seeking response in the absence of any history of primary reinforcement of that response (27)—a canonical test, and measure of the potency, of conditioned reinforcement (28). Once acquired in such an "acquisition-of-a-new-response" procedure, seeking behavior by rats will persist for many weeks being reinforced only by the CS, with the animal never having received the primary reinforcer (e.g., cocaine) for making those responses (27,29). In widely used extinction-reinstatement (30), or increasingly used incubation-of-craving (31) procedures, it is the conditioned reinforcing properties of the CS that underlie "relapse"—i.e., rats learn instrumentally to respond for the CS, acting as a conditioned reinforcer, in the absence of the self-administered drug after either a period of instrumental (not CS) extinction (i.e., extinction-reinstatement) or a period of abstinence when the behavioral impact of the conditioned reinforcer increases with time in abstinence (i.e., incubation of craving).

In our own studies we have focused on the seeking of drugs under second-order schedules of cocaine [following our earliest experiments with Markou on cocaine (32,33)], heroin, alcohol, and high-incentive food reinforcement [see for example (34,35–38)]. It should be emphasized that we were far from the first to identify the utility and explore the use of second-order schedules of drug reinforcement in rats. Pioneering studies in the 1970s by the late Steve Goldberg and colleagues at the National Institute on Drug Abuse (39–41) should be acknowledged for that advance [reviewed in Everitt and Robbins (10)]. These procedures capture many of the features of foraging for natural or drug rewards in the real world because they incorporate delays to primary drug reinforcement that an animal is able to bridge through the mediating effects of drug-conditioned reinforcers. Human subjects seeking cocaine under a second-order schedule of reinforcement in the laboratory also revealed that response-contingent cocaine-associated CSs could maintain behavior even when placebo rather than the drug was ultimately infused (42).

In the procedure established in our lab by graduate student Mercedes Arroyo and Markou (32), which has changed little ever since, rats are initially trained to make instrumental responses for intravenous infusions of cocaine under a continuous reinforcement schedule (fixed ratio 1). We refer to this as "drug taking" to emphasize the low demand simplicity of responding for constantly available drug with no requirement to forage (seek), and that the drug is on board after the first response and therefore affects all subsequent responses. Each infusion is paired with the presentation of a neutral environmental stimulus (usually a light), such that pavlovian association between drug effect and this increasingly salient stimulus occurs. Subsequently, the now light CS is used to reinforce instrumental responses under high ratio demands and the drug is only actually self-administered after, usually, a fixed interval of 15 minutes. Hence, there is not only a tight relationship between responses and CS presentation, but also a weaker relationship between responding and drug infusion. This captures the essence of drug seeking: vigorous responding over delays to reinforcement mediated by the contingent presentation of the CS acting as a conditioned reinforcer (Figure 1A); this is what conditioned reinforcers do in the real world. Each day, rats will work avidly for the CS (usually delivered after every 10 lever presses) and will earn the drug only on completion of the first ratio of 10 responses after the fixed interval (usually 15 minutes) has elapsed; rats accelerate their responding as the interval progresses and the time of infusion becomes imminent (10) (Figure 1). In the