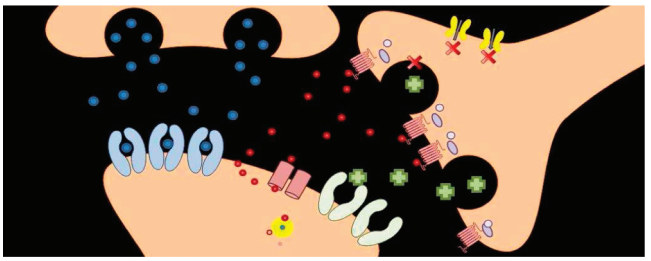
ACS Chemical Neuroscience pubs.acs.org/acschemicalneuroscience Review

Cannabinoid Conditioned Reward and Aversion: Behavioral and Neural Processes

Jennifer E. Murray*,†,‡ and Rick A. Bevins†

†Department of Psychology, University of Nebraska—Lincoln, Lincoln, Nebraska, and ‡Department of Experimental Psychology, University of Cambridge, Cambridge, U.K.

Abstract



The discovery that delta-9-tetrahydrocannabinol (Δ^9-THC) is the primary psychoactive ingredient in marijuana prompted research that helped elucidate the endogenous cannabinoid system of the brain. Δ^9-THC and other cannabinoid ligands with agonist action (CP 55,940, HU210, and WIN 55,212-2) increase firing of dopamine neurons and increase synaptic dopamine in brain regions associated with reward and drug addiction. Such changes in cellular processes have prompted investigators to examine the conditioned rewarding effects of the cannabinoid ligands using the place conditioning task with rats and mice. As reviewed here, these cannabinoid ligands can condition place preferences (evidence for rewarding effects) and place aversions (evidence for aversive qualities). Notably, the procedural details used in these place conditioning studies have varied across laboratories. Such variation includes differences in apparatus type, existence of procedural biases, dose, number of conditioning trials, injection-to-placement intervals, and pretraining drug exposure. Some differences in outcome across studies can be explained by these procedural variables. For example, low doses of Δ^9-THC appear to have conditioned rewarding effects, whereas higher doses have aversive effects that either mask these rewarding effects or condition a place aversion. Throughout this review, we highlight key areas that need further research.

Keywords: Drug addiction, choice behavior, conditioned place preference, marijuana, mesolimbic dopamine, reward

1. Introductory remarks

Isolation of brain cannabinoid (CB) receptors and the endogenous CB compounds, arachidonylethanolamide (anandamide) and 2-arachidonylglycerol (2-AG), as well as the development of exogenous ligands, has enabled a growing body of research into the actions of cannabinoids in the brain and their effects on behavior. Because the primary active ingredient of marijuana is cannabinoidergic and because the prevalence of marijuana use is a global concern, an area of particular interest is how the CB system functions within the brain reward system. Place conditioning is a common and potentially useful task for evaluating the conditioned motivational effects of a drug (*1*, *2*). In this task, the animal (usually a rat or mouse) has a distinct environment (context) repeatedly paired with the drug of interest. There is an alternate environment that differs along some stimulus dimension(s) that is equally experienced, but not paired with the drug. Using this method of Pavlovian conditioning, the conditioned appetitive (rewarding) or aversive effects of a drug can be assessed. A conditioned place preference (CPP) is inferred when in a choice test animals spend more time in an environment that had been previously paired with a drug stimulus compared to an alternate environment. Such an outcome suggests that the drug has some rewarding effects that entered into an association with the paired environment. A conditioned place aversion (CPA) is inferred when animals spend less time in the drug-paired environment; this outcome is taken to indicate an aversive effect of the drug (see later for more detailed discussion).

Similar to other behavioral research, the parameters used in place conditioning studies with CB ligands vary widely across laboratories. With some drugs, these variations in procedural details across laboratories seem to make little difference in the overall conclusion regarding the motivational impact of the drug. As detailed in Tzschentke's (*3*) excellent review of the place conditioning literature, studies with drugs such as cocaine or amphetamine consistently report CPP. The opiate drug heroin produces CPP, whereas

Received Date: January 21, 2010
Accepted Date: February 16, 2010
Published on Web Date: March 10, 2010

DOI: 10.1021/cn100005p | *ACS Chem. Neurosci.* (2010), *1*, 265–278

the opioid antagonist naloxone consistently produces CPA. Alternatively, place conditioning literature involving the cannabinoid system seems to parallel the place conditioning literature with nicotine. That is, reports of no effect, CPA, and CPP with no clear answer yet as to the relevant conditions under which conditioned appetitive or aversive effects will be expressed. With this in mind, the purpose of the present review was to discuss the role of cannabinoids within the reward (motivation) system and to coalesce into one paper the published research on place conditioning with cannabinoid agonists. In doing so, we hoped to identify some critical variables that predict when a cannabinoid agonist may have conditioned appetitive versus conditioned aversive effect. Such information would be important for guiding future research attempting to identify the behavioral and neurochemical processes underlying the conditioned motivational effects of cannabinoid agonists.

2. Endogenous Cannabinoid System

2.1. Receptors

Cannabis has been used for thousands of years for its mood-altering, hallucinogenic, and anesthetic properties. The neurological effects of the drug suggested a central mechanism of action. Delta-9-tetrahydrocannabinol (Δ^9-THC) was isolated as the primary psychoactive component of cannabis (*4*). This compound was then used to help elucidate the CB receptors (*5*). CB receptors have been divided into two groups, CB_1 and CB_2 receptors, on the basis of functionality and distribution. CB_1 receptors are found widely throughout the brain and perform a variety of modulatory functions, whereas the CB_2 receptors have generally been associated with the peripheral and central regulation of the immune system (*6*, *7*, *8*). Furthermore, recent evidence suggests non-CB receptor binding of endogenous (internally produced) and exogenous (externally produced) cannabinoid compounds (*9*, *10*). The current review will primarily focus on the CB_1 receptor because of its purported role involving the rewarding and reinforcing effects of drugs.

The distribution of CB_1 receptors on brain neurons in the striatum was first described using *in vitro* receptor autoradiography with the radioligand [^{3}H]CP 55,940 (*11*). The receptors were initially found in high densities (quantities) in various striatal areas including the caudate putamen and the globus pallidus, as well as in the substantia nigra. The subsequent development of CB_1 antibodies allowed for more specific cellular localization throughout the entire rat brain (*12*). Briefly, a high density of CB_1 receptors was found in the hippocampus, cerebellum, striatum, and substantia nigra. Receptors in the olfactory bulb, piriform cortex, anterior part of the medial forebrain bundle, the cingulate cortex, amygdala, claustrum, and nucleus accumbens were found in moderate density. Finally, CB_1 receptors were found in low density in the thalamus, hypothalamus, periaqueductal gray, pons, medulla, and the area postrema.

Importantly, receptor density does not necessarily indicate signaling strength. CB receptors are G-protein coupled (*13*), meaning the effects of receptor binding are mediated by the second messenger pathways activated by the G-protein. As such, binding can have a different impact depending on localization. For instance, using autoradiography and membrane saturation analyses in male Sprague−Dawley rats, the average number of G-proteins activated per bound CB_1 receptor (i.e., amplification factor) was lowest in the frontal cortex, cerebellum, hippocampus, and striatum (*14*). These are regions with generally high numbers of receptors. Moderate amplification factors were found in the thalamus, brainstem, amygdala, and sensorimotor cortex. Finally, the hypothalamus had the highest amplification factor, an area with low receptor density. These data suggest that areas with low receptor density may enhance signal strength by increasing the impact of G-proteins on subsequent intracellular processes.

The location of these receptors on neurons is important for understanding receptor function. CB_1 receptors have been found to be primarily localized presynaptically on GABAergic neurons as determined by electrophysiological analyses (*15*, *16*). This presynaptic localization suggests a modulatory role of endocannabinoids. Notably, G-protein activation by bound CB_1 receptors reduces Ca^{2+} conductance (*17*) and increases K^+ conductance (*18*). Both of these actions have been linked to a process known as depolarization-induced suppression of inhibition (*19*). As diagramed in Figure 1, activation of presynaptic CB_1 receptors functions to inhibit subsequent neurotransmitter release from that presynaptic terminal (*20*). Postsynaptic endogenous cannabinoid release increases with increased postsynaptic excitation [(*21*) see later]. The subsequent retrograde cannabinoid signaling can attenuate the release of GABA, resulting in less inhibitory input into the synapse and therefore further postsynaptic excitation.

2.2. Ligands

Cannabinoidergic ligands found naturally in the body, including anandamide and 2-AG, are derived from arachidonic acid (*22*−*25*). As displayed in Figure 1, these molecules are synthesized and released in a Ca^{2+} dependent manner following membrane excitation (*21*, *26*). They are then taken back into the cell by anandamide or 2-AG transporters (*26*, *27*) and broken down by anandamide amidohydrolase (*28*) or fatty acid amide

DOI: 10.1021/cn100005p | ACS Chem. Neurosci. (2010), 1, 265–278

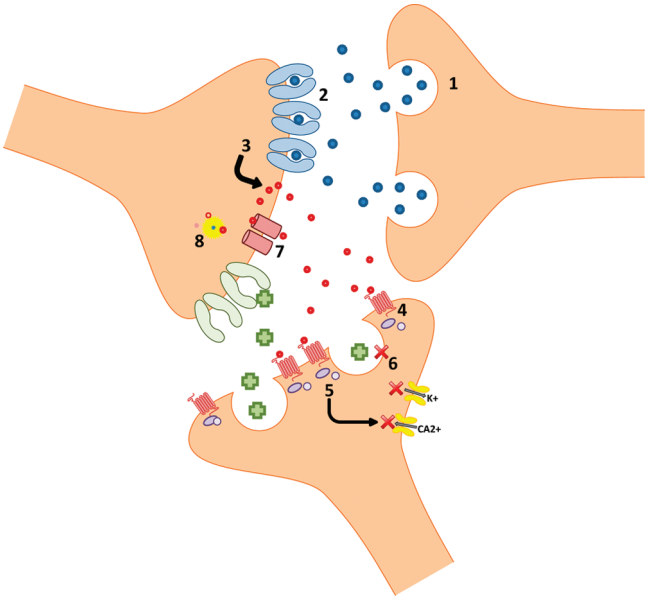


Figure 1. Cellular excitation causes the release of neurotransmitters (1). Neurotransmitters bind to receptors causing postsynaptic excitation (2) that triggers synthesis and nonvesicular release of endocannabinoids (3). Activation of presynaptically localized G-protein coupled cannabinoid receptors typically found on GABAergic neurons (4) signals a reduction in K^+ and Ca^{2+} conductance (5), thereby inhibiting presynaptic GABA release (6). Inhibition of GABA release allows excitatory neurotransmitters to have a greater impact on the postsynaptic membrane. Cannabinoid transporters are responsible for reuptake of the cannabinoids in the synapse (7) where they are broken down by enzymes into their constituent parts (8). More details can be found in the text.

hydrolase (*29*) into constituent parts. These constituent parts can be synthesized back into the signaling molecules again when needed.

Experimentation with chemical analogues of Δ^9-THC yielded a number of potent exogenous CB agonists, including CP 55,940, HU-210, and WIN 55,212-2 (*30*−*35*). Each of these agonists has since been used in behavioral and neurochemical research to help elucidate the localization and role of CB receptors in the brain. For instance, since Δ^9-THC made a poor candidate for radiolabeling, the synthesis of a more potent, more stable ligand was required. CP 55,940 was just such a molecule, and radiolabeled CP 55,940 was instrumental in providing evidence of the CB receptor (*5*). CP 55,940 has also been used in behavioral assays such as drug discrimination (*36*−*38*). HU-210 has been useful in examining the stress response to drug withdrawal (*39*, *40*). In addition to a number of place conditioning studies, WIN 55,212-2 has been used to examine the role of cannabinoids in memory (*41*, *42*). Furthermore, an anandamide transport inhibitor, AM404, has also been used to examine the effect of increased synaptic availability of anandamide on behaviors ranging from place conditioning to cocaine self-administration (*43*−*45*).

3. Drug Reward Circuitry

3.1. Mesolimbic System

There are a number of excellent reviews of the reward system and addiction (*46*−*49*). As such, we will only provide a brief introduction of this system and refer the interested reader to these reviews for more detail. Although the mesolimbic dopamine pathway of the ventral striatum is now perceived as being a small portion of a much larger circuitry responsible for the transition into compulsive drug-taking behavior (*50*), the pathway is generally considered to be associated with the rewarding properties of drugs and the initiation of drug use (*51*). Drug administration and cues predictive of drug administration activate that system (*52*−*57*). The ventral tegmental area (VTA) has dopaminergic projections to the nucleus accumbens (NAcc) and to the prefrontal cortex. Increase in dopamine in the NAcc in particular has been associated with the rewarding effects of abused drugs because prevention of dopamine transmission in this region typically reduces drug self-administration [e.g. ref *58*, but see ref *59*] and conditioned place preference (*60*, *61*). The increased dopamine can come about in a number of ways. For instance, stimulants such as methamphetamine, amphetamine, and cocaine block the reuptake of dopamine in the synapse, resulting in prolonged availability of the neurotransmitter (*62*). Nicotine functions at pre- and postsynaptic sites, modulating activation of the VTA both directly on dopaminergic neurons and by potentiating excitatory glutamate release (*63*, *64*). Additionally, dopaminergic neurons of the VTA receive inhibitory GABAergic input from interneurons in the VTA and from medium spiny neurons of the nucleus accumbens (*65*). Heroin and morphine activate VTA dopamine release into the NAcc via disinhibition of GABA transmission in the VTA (*66*). These GABA neurons are also the purported source for the effects of cannabinoid compounds on the mesolimbic dopamine system (see Figure 1).

3.2. Cannabinoid Modulation

Administration of Δ^9-THC, CP 55,940, HU210, and WIN 55,212-2 dose-dependently enhanced the firing of dopamine neurons in the VTA (*67*−*69*) and increased dopamine concentration in the NAcc shell (*70*) in rat mesolimbic slices and *in vivo*. Further, administration of the CB_1 antagonist SR 141716 (rimonabant) prevented these effects, indicating direct contribution of CB receptor activation in the dopamine enhancement (*67*, *69*−*71*). Similar to the effects of opiates, the mechanism of this increased dopamine release in the NAcc is considered to be modulatory rather than a direct effect on VTA dopamine neurons (*72*). As described earlier, CB_1 receptors are localized presynaptically, typically on

DOI: 10.1021/cn100005p | *ACS Chem. Neurosci.* (2010), *1*, 265–278

GABAergic neurons (*16*). Within the mesolimbic system, there are GABAergic inputs onto VTA dopaminergic neurons that project to the NAcc (*73*). Using a patch-clamp technique, administration of WIN 55,212-2 or CP 55,940 was shown to depress GABA-mediated inhibitory postsynaptic currents via a presynaptic inhibition of GABA release (*74*, *75*). This modulatory effect was due to the inhibition of Ca^{2+} and activation of K^+ channels at presynaptic terminals attenuating further GABA release (*17*, *18*). The reduction of GABA-mediated inhibitory postsynaptic currents on VTA projections to the NAcc resulted in increased transmission of dopamine in midbrain slices that was also blocked by the administration of SR 141716 (*75*). For further discussion of the role of endocannabinoid modulation of neurotransmission with the mesolimbic system, we refer the reader to the following reviews in refs *76*−*79*.

4. Place Conditioning

4.1. Typical Protocol

Environmental or situational cues that reliably co-occur with a drug can acquire the control of drug-related behaviors through Pavlovian conditioning processes (*1*, *80*). Researchers can capitalize on this associative learning to study the appetitive (rewarding) or aversive effects of a drug. As noted in the Introductory Remarks, one widely used task to do so is referred to as place conditioning (*1*−*3*, *81*). Although there are many variations of this method, in a typical place conditioning experiment, animals are exposed to two distinct contexts (e.g., variations in floor texture, wall color, odor, etc.); however, the drug is experienced only in one of the two contexts. When the animal is given a subsequent choice test with unrestricted access to both contexts in a drug-free state, the drug-paired context (conditioned stimulus; CS) now evokes either an approach (conditioned place preference; CPP) or avoidance (conditioned place aversion; CPA) conditioned response (CR) depending on the nature of the previously experienced drug effects (unconditioned stimulus; US).

4.2. Measurement Considerations

Determination of conditioning is typically based on an increase (i.e., CPP) or decrease (i.e., CPA) in the time spent in the paired context relative to the unpaired context at test, the paired context before conditioning (i.e., familiarization session), or to an untreated control. In an apparatus constructed with two compartments, not reporting time spent in the unpaired context is acceptable practice because time spent in the paired context necessarily subtracts from time spent in the unpaired context (*82*). However, many laboratories use three-compartment chambers, in which a center compartment distinct from the other two is used as a discrete starting place for choice tests (*83*). For some researchers, a benefit of having this third compartment is that it provides a novel context, thus detracting from a potential novelty-seeking account of increased time spent in the drug-paired compartment while in the nondrug state (*1*). However, there is some argument against the influence of this effect (*2*). In laboratories using three-compartment chambers, not reporting time spent in the unpaired context or a ratio measure that includes this time is problematic for interpretation. That is, an increase in time spent in the paired context may reflect less time spent in the center compartment rather than a shift from the unpaired to the paired compartment (*84*). If this occurred, there may still be greater time spent in the unpaired than in the paired compartment. Clearly, this is not a conditioned place preference, and no conclusion regarding the conditioned rewarding effects of the drug under study may be made.

4.3. Apparatus and Procedure Bias

When reading a paper on place conditioning, one should be aware of whether the apparatus was constructed in such a way as to bias the behavior of the animal. This bias in rats and mice can be accomplished by using stimuli such as a dark chamber that evokes approach behaviors. Alternatively, using bright illumination or widely space rod bars for flooring can evoke avoidance behaviors (*85*). An important issue with apparatus bias is that the unconditioned bias may shift with repeated exposures which can complicate interpretation of place conditioning results. That is, it becomes unclear whether the drug treatment, the apparatus exposure, or some combination of the two evoked the change in choice behavior. One solution several investigators have tried to solve this problem is to include a vehicle−vehicle control (*86*, *87*). This control allows one to determine how mere apparatus exposure shifts the unconditioned apparatus bias. Unfortunately, this control does not provide an assessment of how drug exposure might interact with these unconditioned biases still leaving some questions regarding the interpretation of any purported place conditioning effect (*1*, *2*).

Another important consideration is whether a biased conditioning procedure was used in the study. This bias refers to how and which compartment was assign to be paired with the drug. A biased procedure is being used if all subjects are assigned to receive the exact same compartment (all drug pairing in white side) or if subjects were assigned based on their behavior in a preconditioning choice test (all drug parings in the initially nonpreferred side). Using the initially preferred compartment could result in a ceiling effect, preventing detection of the development of the conditioned reward. Using the nonpreferred compartment as the paired

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context allows for detection of a change in compartment choice; however, the mechanism of that response becomes unclear. Is time spent in the paired compartment increasing because that compartment now had conditioned rewarding value due to the pairing of the drug effects or because the aversive qualities of the context were decreased by further familiarization? The attenuated aversion could be a result of unconditioned drug effects, but they could also have developed without the influence of the drug, thereby confounding the interpretation of the results. Another bias of the conditioning procedure often occurs when animals are given two conditioning sessions per day. In order to prevent lingering effects of (or possibly withdrawal from) the drug treatment, all animals receive vehicle for the first conditioning session and drug treatment for the second conditioning session of the day. This conditioning protocol confounds drug exposure and training order.

A complete discussion of the implications of procedure and apparatus bias in place conditioning studies is outside the scope of this review. Thus, we refer the reader to some articles that directly examine and/or review the issue in more detail (*1*−*3*, *88*, *89*). However, we would like to echo their general conclusion and ask the reader to keep these issues in mind for the following section that discusses the place conditioning literature with cannabinoids. That is, to obviate any interpretative issues, place conditioning research should use unbiased procedures and balanced apparatus construction.

5. Place Conditioning and Cannabinoid Compounds

Unlike classic psychomotor stimulants such as amphetamine or cocaine that readily condition a place preference at a number of doses and under a variety of conditions, cannabinoid agonists show more mixed results [(*3*, *81*) see earlier]. The extant variation in parameters used by different laboratories to assess place conditioning with cannabinoidergic compounds complicates interpretation and hence conclusions regarding this system. Despite this difficulty, there are a number of experimental variables that appear to impact the outcome of some studies. These variables include the specific compound and its dose, number of conditioning trials, session length, injection-to-placement interval, and pretreatment. Table 1 provides details about Δ^9-THC studies, and Table 2 provides details about other cannabinoid compounds. Figure 2 provides the chemical structures of each of these compounds. The remainder of this review will discuss each of these variables and the evidence for conditioned appetitive or aversive effects of these ligands.

5.1. Drug Specificity

Cannabinoid ligands have distinct specificity and potency. Initial interest in the cannabinoid system was prompted by the psychotropic effects of Δ^9-THC. Subsequently developed synthetic cannabinoids are more efficacious at CB receptors than Δ^9-THC, which is a partial agonist (*90*, *91*). The two primary endogenous cannabinoids in the brain, anandamide and 2-AG, have different efficacies at CB_1 receptors. Anandamide is a partial agonist, whereas 2-AG is a full agonist (*92*, *93*). The synthetic cannabinoids HU-210 and WIN 55,212-2 are full agonists (*90*, *92*). CP 55,940 has been described as a full agonist (*90*) and as a high efficacy partial agonist (*92*) . Finally, the anandamide transport inhibitor, AM 404 selectively blocks the reuptake of anandamide, allowing the signal to last longer in the synapse and effectively serving as an agonist (*43*). Because of these differences in action, behavioral differences in their effects likely exist. As such, generalization of effects across compounds should not be made. Rather, it will be important to test these compounds within a given set of behavioral parameters if firm conclusions are to be made regarding the mechanism. For instance, Cheer et al. (*94*) found CPA in rats with both Δ^9-THC and HU-210 using an unbiased apparatus and procedure. Bortolato et al. (*44*) found similar CPP effects using AM404 and WIN 55,212-2. Mallet and Beninger (*95*), however, found no effect of anandamide, yet Δ^9-THC conditioned a place aversion.

5.2. Drug Dose

One of the primary considerations for behavioral research is the dose of the drug. Very low doses of Δ^9-THC generally have limited rewarding effects and therefore often do not condition an approach response relative to the vehicle. Conversely, high doses appear to include an aversive quality often sufficient to compete with or overshadow any rewarding effects and thus condition an avoidance response. For instance, rats conditioned with 0.015 mg/kg Δ^9-THC display choice behavior during testing indistinguishable from that of the vehicle (*96*). Doses from 0.075 to 0.75 mg/kg Δ^9-THC, however, appear to condition a place preference; 1 and 3 mg/kg show no difference from vehicle, yet 6 mg/kg Δ^9-THC conditions an aversion (*96*). Time between training sessions appears to interact with drug dose and has an impact on the conditioned effects of Δ^9-THC. When rats were trained with 24 h between sessions, 1 mg/kg Δ^9-THC had no effect, and 2 and 4 mg/kg Δ^9-THC conditioned a place preference (*97*). However, when there were 48 h between sessions, 1 mg/kg conditioned a place preference, and 2 and 4 mg/kg Δ^9-THC conditioned a place aversion. Thus, allowing a longer wash-out period between training sessions shifted the dose−effect curve to the left. The importance

DOI: 10.1021/cn100005p | ACS Chem. Neurosci. (2010), 1, 265–278

Table 1. Δ9-THC Place Conditioning Study Details

Species	Chamber Compartments	Bias Design	Bias Chamber	veh-veh Control	Number of Trials	Time in minutes Session	Time in minutes Test	Route	IPI	Dose (mg/kg)	Additional Treatments	Result	Reference
Mice	3	UNB	UNB	Yes	5	45	20	IP	immed	1	1 inj 24 hrs	CPP	86
										1	–	NS	
										5		CPA	
	3	UNB	UNB	Yes	5	45	20	IP	immed	1	1 inj 24 hrs	CPP	115
										5	–	CPA	
	3	?	?	Yes	4	45	20	IP	immed	0.3	–	NS	116
	3	UNB	?	Yes	4	30	30	IP	15	1	1 inj 24 hrs	NS	110
										3	1mg/kg 24 hrs	NS	
	3	UNB	?	Yes	5	45	20	IP	immed?	1	1 inj 24 hrs	CPP	117
	3	UNB	UNB	Yes	5	45	20	IP	immed?	0.3	–	NS	118
										1	1 inj 24 hrs	CPP	
	2	UNB	?	Yes	5	45	20	IP	immed	1	–	NS	103
										5		CPA	
	3	UNB	?	Yes	5	45	20	IP	immed	1	–	NS	87
										5	–	CPA	
										1	1 inj 24 hrs	CPP	
										5	1 inj 24 hrs	NS	
	3	UNB	?	Yes	5	45	20	IP	immed	5	–	CPA	102
	3	BIAS	UNB	Yes	3	30	18	IP	immed	0.5	–	NS	104
										1		NS	
										2.5		NS	
										5		NS	
										10		NS	
										20		CPA	
	3	?	?	Yes	5	45	20	IP	immed	1	1 inj 24 hrs	CPP	101
										5	–	CPA	
	3	UNB	BIAS	Yes	5	30	15	IP	immed	1	–	NS	105
										10		CPA	
Rats	2	BIAS	?	Yes	3	30	15	IP	10	0.01	–	NS	119
										0.1		CPP	
										1		NS	
										2		NS	
	3?	?	?	Yes	4	30	15	IP	immed	0.3	–	CPP	120
	2	UNB	?	Yes	4	30	15	IP	10	0.015	–	NS	96
										0.075		CPP	
										0.15		CPP	
										0.37		CPP	
										0.75		CPP	
										1		NS	
										3		NS	
										6		CPA	
	3	BIAS	BIAS	Yes	4	30	18	IP	30	0.1	–	NS	95
										0.5		NS	
										1		CPA	
										1.5		CPA	
										2		NS	
										4		NS	
										8		NS	
	3	UNB	UNB	Yes	3	30	15	IP	20	1	–	NS	97
										2		CPP	
										4		CPP	
										0.5	48hr b/t sessions	NS	
										1		CPP	
										2		CPA	
										4		CPA	
	2	UNB	UNB	Yes	2	30	20	IP	immed	1.5	48hr b/t sessions	NS	121
										15		CPA	
	2	UNB	?	No	3	30	15	IP	5	0.2	–	CPA?	122
										0.4		CPA?	
										0.75		CPA?	
										1.5		CPA?	
	2	UNB	UNB	Yes	3	10	10	IP	10	1.5	–	CPA	94

of this wash-out period to the motivational effects of Δ^9-THC and the underlying processes deserve more empirical attention. Another consideration with regard to drug dose is the species of the subject. Mice

DOI: 10.1021/cn100005p | ACS Chem. Neurosci. (2010), 1, 265–278

Table 2. CB Agonists and Transporter Inhibitor Place Conditioning Study Details

Drug	Species	Chamber Compartments	Bias Design	Bias Chamber	veh-veh Control	Number of Trials	Time in minutes Session	Time in minutes Test	Route	IPI	Dose (mg/kg)	Additional Treatments	Result	Reference
ANA	Rats	3	BIAS	BIAS	Yes	4	30	18	IP	5	0.031 0.125 0.313 0.5 2 8 16	PMSF*	NS NS NS NS NS NS NS	95
		*2.0 mg/kg phenylmethylsulfonyl fluoride IP 40 min before all conditioning sessions (anandamide and vehicle)												
		2	BIAS	BIAS	Yes	3	20	15	IV	immed	0.03 0.1 0.3 3	–	NS NS NS NS	100
											0.03 0.1 0.3 3	URB597*	NS NS CPA CPA	
		*0.3 mg/kg IP 40 min before all conditioning sessions (anandamide and vehicle)												
HU-210	Rats	2	UNB	UNB	Yes	3	10	10	IP	10	0.02 0.06 0.1	–	CPA CPA catalepsy	94
CP	Rats	3	UNB	UNB	Yes	4	30	20	IP	immed	0.01 0.1	–	CPA CPA	37
		2	BIAS	BIAS	Yes	4	30	15	IP	10	0.01 0.02 0.03 0.04	–	NS CPP NS NS	82
WIN	Mice	3	BIAS	BIAS	Yes	4	60	15	IP	immed	0.01 0.05 0.1 0.5 1	–	NS CPP NS NS NS	123
		2	BIAS	UNB	Yes	5	45	20	IP	immed?	0.1 1	–	NS NS	41
											0.1 1	1 inj 24 hrs	CPP NS	
	Rats	4	BIAS	BIAS	Yes	5	30	20	SC	immed	0.4 2 10	extended alcohol	CPA CPA CPA	124
		2	BIAS	BIAS	Yes	6	60	15	IP	immed	1 1	– enriched	NS CPP	44
		2	BIAS	BIAS	Yes	3	20	15	IV	immed	0.015 0.05 0.15 0.3	–	NS CPA CPA CPA	100
		2	BIAS	?	Yes	4	30	20	SC	15	0.003 0.01 0.03 0.1 0.3 1	–	NS NS NS NS CPA CPA	125
		3	?	?	Yes	4	25	15	IP	immed	0.125 0.25 1.25 2.5	–	NS CPA CPA CPA	84
		3	BIAS	?	Yes	4	30	15	IP	10	0.1	–	NS	83
AM404	Rats	2	BIAS	BIAS	Yes	6	60	15	IP	immed	1.25 2.5 5 10	–	NS NS NS NS	44
											1.25 2.5 5 10	enriched	NS CPP NS NS	

generally have a higher rate of drug metabolism (*98*). As such, mice are typically treated with higher doses than rats. For instance, in place conditioning studies using Δ^9-THC, mice were treated with a range of 0.3 to 20 mg/kg, whereas rats were treated with 0.01 to 8 mg/kg (see Table 1). The lowest effective dose (producing significant CPP or CPA) in mice was 1 mg/kg (*86*); in rats, that dose was more than ten times lower, 0.075 mg/kg (*96*).

5.3. Temporal Considerations

The temporal relationship between the drug effects and exposure to the to-be-paired compartment during

DOI: 10.1021/cn100005p | *ACS Chem. Neurosci.* (2010), *1*, 265–278

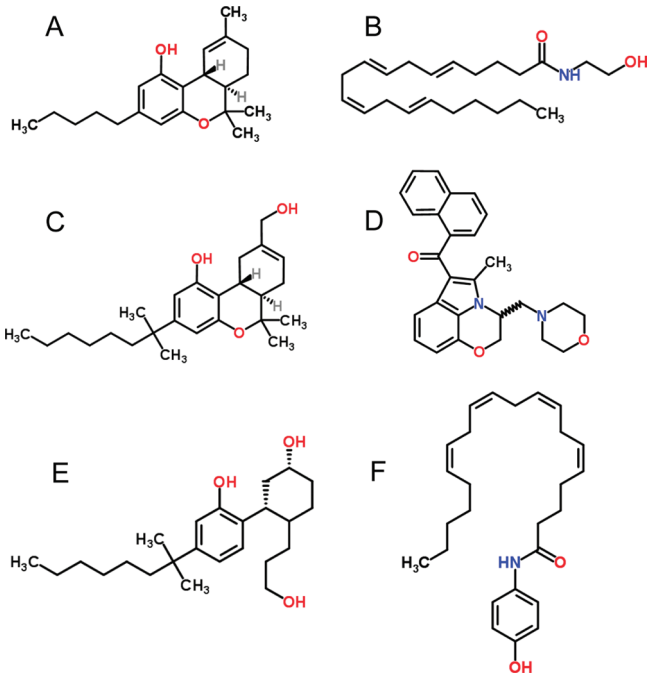

Figure 2. Structures of Δ^9-THC (A), anandamide (B), HU-210 (C), WIN 55,212-2 (D), CP 55,940 (E), and AM404 (F).

the conditioning phase appears to be an important variable in the cannabinoid place conditioning literature. Variation across studies in this relationship between the CS (paired compartment) and the US (i.e., relevant drug effects) might help explain some of the discrepant results. In all conditioning tasks, there is a temporal window in which the US must occur for learning (i.e., CS−US association) to occur. Relationships in which the CS onset occurs before the US onset but with some overlap tends to be more conducive to conditioning than a relationship where the US occurs before or well after the CS (*80*). In the case of place conditioning research with drug USs, drugs have many physiological effects, and the duration and peak of these effects may differ. Because drug effects are variable due to pharmacokinetic and pharmacodynamic reasons, careful consideration should be given to such factors in the design of the experiment. For instance, if a compound is metabolized quite quickly (e.g., anandamide), then conditioning sessions should be shorter so that compartment exposure does not extend well beyond that of the drug effects (*99*). Allowing this may weaken conditioning through a process called extinction (*80*). Alternatively, an enzyme inhibitor can be used to slow ligand breakdown to extend the length of drug effects (*95*, *100*).

In addition, the more an US is paired with the CS, the stronger the conditioned association (*80*). The Maldonado group used this idea to their advantage by extending the time of typical conditioning. In their studies that examined 5 mg/kg Δ^9-THC, mice acquired a CPA [(*86*, *87*, *101*, *102*) see also (*103*)]. Each of these studies used 5 conditioning trials and 45-min sessions. However, when three 30-min sessions had been used by the group instead, there was no conditioned effect (*104*). This latter study had less time in the compartment on each placement, as well as fewer conditioning trials; both variables are important for learning. Although comparing across studies is fraught with difficulties, there are some basic ideas that can be extrapolated and suggest an important avenue for future parametric research.

Similar to the effects of session length, with repeated pairings the strength of the conditioned effect increases. Therefore, conditioning with only a few trials may not be sufficient to observe conditioning that would be expressed with a greater number of trials (i.e., environment−drug pairings). Again, taking the necessary caution of comparisons across studies, mice conditioned with Δ^9-THC in five 30-min sessions developed a CPA at 10 mg/kg as shown in a 15-min test (*105*). However, mice only conditioned for three 30-min sessions did not show a conditioned effect at 10 mg/kg but did show a CPA at 20 mg/kg (*104*). This pattern suggests that the increased number of trials was required for conditioning to be sufficiently strong before being expressed as conditioned avoidance on the test day. With repeated drug exposure, there is also the potential for tolerance and sensitization to the drug effects. Tolerance refers to a gradual decrease in the effects of the drug; sensitization refers to an increase in the drug effects with repeated exposure. Sensitization and tolerance to the effects of Δ^9-THC have been reported (*106*−*109*). Since increased pairings were required for the development of CPA, an alternative account suggests that sensitization of the aversive properties may have developed.

As the discussion in this section implies, the injection-to-placement interval (IPI) would have an impact on the extent of place conditioning. Thus, allowing time to pass following the injection of the drug establishes a different temporal relationship between the compartment CS and the drug US than immediate placement. Along these lines, CPA in rats developed at two doses of CP 55,940 using an immediate IPI (*37*), but an intermediate dose of CP 55,940 conditioned a place preference when a 10 min IPI was used (*82*). Furthermore, when rats had a longer IPI using Δ^9-THC (*95*), the aversive properties of the drug were conditioned at lower doses than when rats had a shorter IPI (*96*). Perhaps the early effects of CP 55,940 are aversive and lengthening the IPI allows those effects to diminish before the start of conditioning. Conversely, perhaps the longer IPI with Δ^9-THC allows the early rewarding effects to diminish, allowing conditioning to occur with the aversive effects of the

DOI: 10.1021/cn100005p | *ACS Chem. Neurosci.* (2010), *1*, 265–278

drug. Of course, there were other variables that differed between these studies (i.e., chamber/placement bias and length of test session); therefore, a final conclusion will need to await the conduction of the appropriate parametric studies.

5.4. Pretreatment Effects

Drug pre-exposure purportedly attenuates the unconditioned aversive effects of the compound and hence reveals CPP upon subsequent training. For instance, mice given 1 mg/kg Δ^9-THC in the home cage 24 h before beginning conditioning developed CPP, whereas mice not pretreated with Δ^9-THC did not develop CPP or CPA (*86*, *87*, *110*). Furthermore, pretreatment can attenuate the aversive effects of Δ^9-THC. Mice given 5 mg/kg Δ^9-THC in the home cage before conditioning did not develop the CPA shown by mice not given pretreatment (*87*). These findings may be due to the development of some tolerance to initial aversive drug effects that are experienced in the home cage rather than in the conditioning chamber. Considering the consistent results of the Maldonado group, there is certainly clear support for the efficacy of pre-exposure attenuating some of the aversive properties of Δ^9-THC in place conditioning studies.

6. Concluding Comments

Place conditioning is useful in determining the conditioned rewarding and/or aversive effects of cannabinergic compounds. However, as stated previously, there is much variability in the place conditioning apparatus and protocol across laboratories studying the conditioned appetitive or aversive effects of cannabinoids. As detailed in this review, such variability likely affects the outcome of the study and indicates a real need for careful parametric research on key factors that might affect conditioning with a particular ligand (dose, session length, number of trials, injection-to-placement interval, etc.). As recommended here and elsewhere (*2*, *88*, *89*), place conditioning studies should avoid procedural or apparatus biases. If achieved, then the field will be better able to advance and have a more coherent picture of the neurochemical system mediating the conditioned motivational effects of cannabinoids. Although the current review was focused on the rewarding and aversive properties of cannabinoids, this system has a much broader applicability. Cannabinoids have been shown to have a role in a variety of behavioral processes; these include pain (*111*), appetite, energy, nausea (*112*, *113*), and stress and mood (*114*). A better understanding of each of these effects and how they may interact with each other will no doubt benefit therapeutic outcomes across these areas.

Author Information

Corresponding Author

*To whom correspondence should be addressed. Department of Experimental Psychology, Downing Street, University of Cambridge, Cambridge, CB2 3EB, U.K. E-mail: jem98@cam.ac.uk.

Funding Sources

While preparing the manuscript for publication, J.E.M. was supported by NIDA F31 DA025399 and MRC 9536855 (awarded to B. J. Everitt), and R.A.B. was partially supported by DA018114 and DA023591.

Abbreviations

2-AG, 2-arachidonylglycerol; ?, uncertain/not stated; Δ^9-THC, delta-9-tetrahydrocannabinol; ANA, anandamide; CB, cannabinoid; CP, CP 55,940; CPA, conditioned place aversion; CPP, conditioned place preference; CR, conditioned response; CS, conditional stimulus; GABA, gamma-aminobutyric acid; hrs, hours; immed, immediate; inj, injection; IP, intraperitoneal; IPI, injection-to-placement interval; IV, intravenous; NAcc, nucleus accumbens; NS, not significant; SC, subcutaneous; UNB, unbiased; veh, vehicle; VTA, ventral tegmental area; WIN, WIN 55,212-2.

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