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Dopamine–Glutamate Interactions
in Reward-Related Incentive Learning

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1. INTRODUCTION

Extensive evidence implicates the neurotransmitter dopamine (DA) in reward-related incentive learning (for reviews, *see* refs. *1–13*). DA projections to the nucleus accumbens (NAc; refs. *14–17*), striatum *(18)*, amygdala *(19)*, and medial prefrontal cortex (mPFC; ref. *20*) have been shown to be involved. In recent years, researchers have begun to focus on the neurochemical mechanisms underlying the role of DA in learning and significant advances have been made *(21–23)*. Many data suggest that DA afferents interact with glutamatergic (Glu) afferents common to the same cell when reward-related learning occurs *(see* ref. *22)*. Results further suggest that a number of signaling molecules activated by Glu and DA synaptic transmission interact to bring about short-term and long-term alterations that mediate the neurochemical and structural changes that form the basis of reward-related incentive learning *(see* ref. *22)*. In this chapter, we will review some of the studies examining the role of DA and especially Glu neurotransmission in reward-related learning. This will be followed by a discussion of evidence that provides a basis for understanding the DA–Glu interactions and the signaling pathways that mediate the effects of reward on behavior. Finally, the role of Glu in reward-related learning will be considered from the point of view of this evidence.

2. GLUTAMATE AND REWARD-RELATED LEARNING

In the following subheadings, the role of Glu in reward will be reviewed. Each section will begin with a brief discussion of the role of DA in the phenomenon under consideration. There have been many studies of DA manipulations and no attempt will be made to exhaustively review those studies. Instead, representative studies will be presented to provide a background of the role of DA against which the Glu results can be viewed.

2.1. Glutamate and Appetitive Learning: Acquisition and Expression of Conditioned Approach Responses

When a neutral stimulus is paired with a rewarding stimulus such as food, animals begin to make approach responses to the neutral stimulus *(24)*. This type of learning can

be seen in the increase in approach responses to the food tray in operant chambers after food has been delivered there on a number of occasions. DA receptor antagonists decrease this type of learning. For example, the D2-like DA receptor antagonist pimozide impaired the acquisition of conditioned approach responses to a food magazine signaled by a light *(25)*. In a number of related studies, conditioned approach responses of animals that had received conditioning prior to drug treatment also were impaired by pimozide or the related D2-like DA receptor antagonists metoclopramide and haloperidol but not thioridazine *(26–28)*. Dickinson et al. *(29)* paired food with an auditory conditioned stimulus presented to rats pretreated with pimozide or the mixed D1- and D2-like DA receptor-antagonists α-flupenthixol and then presented the auditory stimulus while the animals were lever pressing in a drug-free state. They found that the auditory stimulus increased responding in control rats but not in those that had been treated with a DA receptor-antagonist during conditioning; this suggested that drug treatments had blocked acquisition by the auditory stimulus of the ability to energize responding. A. Phillips et al. *(26)* showed that the maximal impairment produced by pimozide or haloperidol was not immediate but had a gradual onset with repeated presentations of the conditioned appetitive stimulus over test trials; this observation suggested that, although DA was necessary for the maintenance of responding to conditioned stimuli, once they were established, these conditioned stimuli may have been temporarily resistant to the effects of DA receptor blockade. Thus, studies using systemic drug administration implicate DA in the acquisition and long-term maintenance of conditioned approach responses to conditioned appetitive stimuli but, once established, this conditioning may be transiently resistant to the effects of DA receptor antagonists.

DA in the NAc, especially the core region, has been shown to play a role in the acquisition and the expression of conditioned approach responses. Thus, NAc core injections of the D1-like DA receptor antagonist SCH 23390 *(30)*, α-flupenthixol *(31)*, or NAc DA depletions with 6-hydroxydopamine impaired both acquisition and expression of conditioned approach responses *(32)*. The latter study found milder effects on expression vs acquisition suggesting again that once conditioning had taken place, it may have been transiently resistant to the effects of decreased DA neurotransmission. In one study, the mPFC was implicated: Baldwin et al. *(33)* reported that mPFC infusions of SCH 23390 impaired acquisition and expression of conditioned approach; however, higher doses were required to impair expression. Like studies using systemic administration of DA receptor antagonists, those using intra-NAc or -mPFC injections showed that DA plays a critical role in acquisition and expression of conditioned approach responses but that expression is somewhat resistant to the drug effects.

Glu *N*-methyl-D-aspartate (NMDA) receptors have been implicated in conditioned approach. In all of the studies reviewed in this paragraph, NMDA receptor antagonists were found to impair acquisition but not expression of conditioned approach responses. Impairments were seen following chronic intracerebroventricular (icv; ref. *34*), unilateral or bilateral basolateral amygdala (BLA; refs. *35* and *36*) or NAc core (but not shell; refs. *31, 36,* and *37*) or bilateral mPFC infusions of the NMDA receptor antagonist 2-amino-5-phosphonovaleric acid (AP5; ref. *36*). Injections of AP5 into the dorsal or ventral subiculum were without effect *(36)*. Di Ciano et al. *(31)* studied discriminated approach to a lever that was extended to signal the delivery of a food pellet; they showed that intra-NAc core injections of the α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid

(AMPA)/kainate receptor antagonist (3S,4aR,6R,8aR)-6-[2-(1(2)H-tetrazole-5-yl)-ethyl]-1,2,3,4,4a,5,6,7,8,8a-decahydroiso-quinoline-3-carboxylic acid (LY293558) did not block acquisition of approach and contact responses but impaired discrimination so that approach and contact responses were seen to levers that signaled either food or no food. Results implicate NMDA receptors in the NAc core, BLA, and mPFC in the acquisition but not expression of conditioned approach responses. AMPA/kainate receptors in the NAc core may play a role in discrimination learning.

Synergistic effects of DA D1-like and Glu NMDA receptors have been found in the NAc core and mPFC. Thus, coinjections of subthreshold doses of SCH 23390 plus AP5 blocked acquisition but not expression of conditioned approach responses *(30,33)*. Results suggest that both DA D1-like receptors and Glu NMDA receptors in NAc core and mPFC participate in the learning that underlies the acquisition of conditioned approach responses.

2.2. Glutamate and Appetitive Learning: Lever Pressing Tasks

2.2.1. Glutamate and the Acquisition of Lever Pressing for Food

One form of appetitive instrumental learning is the acquisition of lever pressing for food in rats. In this paradigm, food-deprived rats learn that food is available from a food hopper in an operant testing chamber outfitted with a lever. Once rats have been fed in this situation, they become more active and often sniff, bite at, and manipulate environmental stimuli associated with food. Occasionally this activity leads to a downward deflection of the lever that is programmed to deliver a food pellet to the food hopper. Rats tend to return to environmental stimuli that were encountered just before the presentation of a rewarding stimulus and this tendency leads the rats to further manipulate the lever and to attain further rewards. This process brings about instrumental learning evidenced by the rat's repeated lever pressing.

It is well-known that DA is necessary for this form of learning. Thus, treatment with the DA D2-like receptor blocking agent pimozide dose-dependently attenuated the acquisition of lever pressing for food *(25,38)*. Tombaugh et al. *(25)* found that animals trained to lever press and then given pimozide showed little effect of the drug in two 22.5-min sessions. This result makes it difficult to attribute the failure of rats to learn to lever press while treated with pimozide to motor effects of the drug; clearly, previously trained rats, when treated with pimozide, can lever press. Thus, the DA receptor antagonist blocked acquisition learning of appetitive instrumental conditioning.

It has also been shown that DA in the NAc and the mPFC is important for the acquisition of appetitive instrumental conditioning. Rats were given daily 15-min sessions in an operant chamber outfitted with two levers, one of which produced a food pellet when depressed. Normal rats generally learned to lever press in the third session and rates increased over subsequent sessions. Smith-Roe and Kelley *(30)* showed that intra-NAc injections of the DA D1-like receptor antagonist SCH 23390 impaired acquisition of lever pressing in this paradigm. The dose of SCH 23390 that impaired acquisition of lever pressing also decreased locomotor activity and increased the average duration of feeding bouts in a 15-min test. However, it did not affect total amount eaten. The authors argued that SCH 23390 did not alter motivation to eat but could not rule out the possibility that the effect of injection of this drug into NAc on lever press acquisition was related to its motor effects.

In a further study from Kelley's laboratory, SCH 23390 injected bilaterally into the mPFC dose-dependently decreased appetitively motivated lever press acquisition (33). In this study, locomotor activity and feeding were not affected by doses of SCH 23390 that impaired lever press acquisition. Results implicated NAc- and mPFC-DA in the acquisition of lever pressing for food.

Kelley and coworkers have extensively evaluated the role of NMDA receptors in this paradigm. They compared the effects of the NMDA receptor antagonist AP5 injected into the NAc core or shell. Injections into both regions impaired lever press acquisition but AP5 was more potent in the core than in the shell. AP5 did not significantly alter locomotor activity or feeding at doses that impaired operant response learning (37). Results demonstrate the requirement of NMDA receptors in NAc for the early stages of learning.

Baldwin et al. (36), in another study from A. Kelley's lab, investigated the effects of bilateral AP5 injections into NAc core, BLA, dorsal subiculum, vental subiculum, or mPFC on lever press acquisition. Learning was impaired by injections into the NAc core, replicating the effects of Kelley et al. (37). Learning was also impaired by injections into the BLA or mPFC but not into the dorsal or ventral subiculum. BLA or mPFC injections of AP5 did not significantly affect locomotor activity or feeding. Results implicate not only NMDA receptors in the core of NAc but also those in BLA and mPFC in instrumental response learning.

Some studies have used systemic injections of Glu agents. One evaluated the effects of glutamic acid diethyl ester (GDEE) on lever press acquisition; GDEE is a nonselective excitatory amino acid (EAA) receptor antagonist. Consistent with the central injection studies reviewed above, Freed and Wyatt (39) found a dose-dependent impairment. The noncompetitive NMDA receptor antagonist ketamine similarly impaired lever press acquisition in a dose-dependent manner; in related experiments, open field performance was unaffected (40). Some studies required rhesus monkeys to learn a new sequence of pressing four levers for food each day and showed that treatment with the noncompetitive NMDA receptor antagonists dizocilpine (aka MK-801) impaired performance (41). Clissold et al. (42) showed that the competitive NMDA receptor antagonist (±)-3-(2-carboxypiperazin-4-yl)propyl-l-phosphonic acid [(±)-CPP] or the noncompetitive NMDA receptor antagonists dizocilpine impaired acquisition of a repeated discrimination lever pressing task; phencyclidine (PCP) was without effect. These studies support a role for NMDA receptors in the acquisition of lever pressing for food.

Mélan et al. (43) found that BALD/c mice that had been partially trained to lever press for food showed a spontaneous improvement of performance 24 h later. If they were injected with an NMDA receptor antagonist immediately after the acquisition session, they did not show this effect. Thus, γ-L-glutamyl-L-aspartate (γ-LGLA) or (±)-CPP dose-dependently eliminated the spontaneous improvement effect. In a related study, post-training icv injections of AP5 eliminated the performance improvement effect in mice (44). Results implicated NMDA receptors in acquisition of lever pressing and were consistent with the findings from A. Kelley's lab that injections of AP5 into the NAc core, BLA, or mPFC impaired learning.

The role of metabotropic glutamate receptors (mGluRs) has also been investigated. Mathis and Ungerer (45) found that posttraining icv injections of the group I and II mGluR antagonist α-methyl-4-carboxyphenylglycine (MCPG) dose-dependently blocked

the spontaneous improvement effect observed 24 h after training in mice. Coadministration of either the group I and II mGluR agonist (1S,3R)-1-aminocyclopentane -1,3-dicarboxylic acid (ACPD), the group I mGluR agonist (R,S)3,5-dihydroxy-phenylglycine (DHPG), or the group II mGluR agonist (1S,2S,5R,6S)- 2-aminobicyclo [3.1.0]hexane-2,6-dicarboxylate monohydrate (LY354740) reversed the impairments produced by MCPG (for a review of these studies and a discussion of the role of NMDA and mGluRs in consolidation of memory, *see* Ungerer et al. ref. *46*). The group I category of mGluRs includes mGluR5 *(47)*; mGluR5 null mutant mice or wildtype mice treated with the mGluR5 antagonist 2-methyl-6-(phenylethynyl)-pypiridine (MPEP) were not impaired in acquiring a lever press response for food *(48)*. Results suggest that group I and II mGluRs are involved in the acquisition of lever pressing for food but that the group I category receptor mGluR5 is not involved; that leaves mGluR1(the remaining member of the group I category) and mGluR2 and 3 (members of group II) as candidates for a role in the learning that underlies acquisition of lever pressing for food.

Smith-Roe and Kelley *(30)* and Baldwin et al. *(33)* identified doses of SCH 23390 and AP5 that were ineffective on their own when injected into the NAc core or mPFC during acquisition training. Coadministration of these doses together produced a significant impairment of lever press acquisition without affecting food intake or locomotion. Results suggest that coactivation of DA D1-like receptors and Glu NMDA receptors in NAc and mPFC plays a critical role in appetitive instrumental learning.

2.2.2. Glutamate and the Expression of Lever Pressing for Food

The effects of DA or Glu receptor antagonists on instrumental behavior can be tested during acquisition, as reviewed in the previous subheading, or during expression following acquisition when the behavior is well established. Numerous studies have shown that lever pressing for food is initially resistant to the effects of DA receptor antagonists; however, with continued testing under the influence of these agents, established responding gradually declines, showing a pattern that resembles that seen during extinction (when food reward no longer is presented following lever press responses). In one of the earliest such studies, Wise et al. *(49)* trained rats to lever press for food. On subsequent days, well-trained rats were injected with pimozide prior to testing. On the first day, drug-treated rats responded almost as frequently during a 45-min session as they had during training. With repeated testing while under the influence of pimozide on subsequent days responding declined. Related studies showed that when intrasession response rates were evaluated over time, they also declined in an extinction-like manner *(50–52)*. It has also been shown that systemic injections of SCH 23390 produce a day-to-day decline in responding for food reward *(53)*. Results suggested that DA acting at both D1- and D2-like receptors is critical for the maintenance or expression of responding in well-trained animals but when drug treatments commence, there is an initial period during which responding is resistant to their effects.

Some studies have sought to identify DA terminal regions that may be critical for the expression of operant responding for food. Beninger and Ranaldi *(18)* and Beninger et al. *(54)*, for example, showed that bilateral injections of the DA receptor antagonist α-flupenthixol into the dorsal caudate nucleus, but not into the NAc, central nucleus of the amygdala, or sensorimotor cortex, produced a within-session decline in responding resembling that seen in extinction. Injections of the inactive isomer *trans*-flupenthixol were without significant effect. G. Phillips et al. *(55)* showed similar effects with dorsal

caudate injections of the DA D2-like receptor antagonist sulpiride. Results suggest that the expression of lever pressing for food in previously trained rats depends on intact neurotransmission at both D1- and D2-like DA receptors and that the caudate nucleus may be the brain region critical for this effect.

In her studies of the effects of intracranial injections of AP5 on the acquisition of lever pressing for food and nose poking into the feeder, Kelley and colleagues used the following protocol (all sessions were 15 min in duration): four sessions preceded by intracranial injections; five sessions with no injections; one session preceded by intracranial injections. The final session provided information about the effects of treatments on expression of the lever pressing response once it was established. They found that an intra-NAc core or shell dose of AP5 that impaired acquisition of responding for food had no significant effect on response expression in trained rats tested in one 15-min session *(36,37)*. Similar results were found with BLA and mPFC injections of AP5 *(36)*. Thus, trained operant responses seemed to be resistant to the effects of centrally injected NMDA receptor antagonists when responding was assessed for one session.

Freed and Wyatt *(39)*, who showed that systemic GDEE impaired acquisition of lever pressing for food *(see* Subheading 2.2.1.), also tested the effects of this nonspecific EAA receptor antagonist on the expression of learned lever pressing 2 d and approx 2 wk after acquisition training. GDEE had no significant effect on the performance of the learned operant.

Systemic dizocilpine failed to affect established lever pressing for food *(56,57)*. In the same report, Shoaib et al. *(56)* found that (+)-3-amino-1-hydroxy-pyrrolid-2-one [(+)-HA966], a partial agonist at the glycine site that acts as a functional antagonist of the NMDA receptor complex, produced a decrease. When dizocilpine was infused via sucutaneous minipumps during performance of a well-trained operant lever press reinforced according to a fixed ratio (FR) 30 schedule, a dose-dependent decrease in responding was observed; this effect showed tolerance with repeated testing for 10 d *(58)*. Poling et al. *(59)* and Hudzik and Slifer *(60)* similarly showed a dose-dependent decrease in FR responding by dizocilpine or the NMDA receptor antagonists PCP or (+)-*N*-allylnormetazocine (NANM) with acute systemic dosing. Hudzik and Slifer *(60)* assessed FR 10 responding in the context of a complex multiple schedule with one of the components being a differential reinforcement of low response rates (DRL) 10-s. They found that doses of the NMDA receptor antagonists that produced decreases in FR 10 responding produced increases in DRL 10-s responding. Increases in DRL 10- or 15-s responding similarly were reported following PCP, dizocilpine, NANM, or (±)-CPP but not the noncompetitive NMDA antagonist ifenprodil *(59,61,62)*. These observations made it difficult to attribute the effects of the NMDA receptor antagonists on FR responding to possible motor impairments. In a related study, Genovese and Lu *(63)* showed that dizocilpine dose-dependently decreased FR 20 rates and increased fixed interval (FI) 2-min rates on a multiple schedule. They also assessed repeated testing under the influence of the drug and observed tolerance, in agreement with the findings of Wessinger *(58)*. Other studies evaluated rates and maxima on progressive ratio schedules in rhesus monkeys; both were dose-dependently decreased by dizocilpine *(41)*. Thus, some results showed that systemic doses of most NMDA receptor antagonists dose-dependently decreased continuously reinforced or FR responding and increased DRL or FI responding; others showed no effect of systemic dizocilpine.

The results from many systemic administration studies showing that Glu receptor antagonists decrease the expression of lever pressing for food are not in agreement with those from central-injection studies showing no effect. However, a number of differences between the two sets of studies should be noted. One relates to the doses tested. Thus, the central-injection studies identified a dose of AP5 that impaired lever press acquisition and then used that dose in the test of expression. By contrast, with one exception, the systemic administration studies did not evaluate acquisition. Thus, it is possible that the doses that were observed to affect established operant responding were higher than those that would have affected acquisition. The one exception is the study by Freed and Wyatt (39) and when these researchers tested the effects of an acquisition-impairing dose of GDEE on expression, they observed no effect.

Pierce et al. (57) evaluated the effects of the AMPA/kainate receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX) on established operant responding for food. They observed a significant decrease. Results suggest that AMPA/kainate receptors may be involved in the expression of lever pressing for food.

In their study showing that subthreshold doses of AP5 and SCH 23390 when given together into NAc synergized to produce an impairment of acquisition, Smith-Roe and Kelley (30) found that the same treatment also caused a significant reduction in established responding; however, responding was still substantially above the level seen in untrained rats, showing some resistance to the effects of the drug treatment. In their related study examining the effects of mPFC co-injections of subthreshold doses of AP5 and SCH 23390, Baldwin et al. (33) found no effect of the coinjections on well-trained lever pressing.

A recent study by Hauber et al. (64), although using a different behavioral paradigm, might be relevant here. They trained rats in a reaction time (RT) task. The rats were presented with a signal to press a lever and then waited (200–800 ms) for an additional signal to release the lever; if they released the lever within a critical amount of time (100–1000 ms from signal onset) they received reward. There were two reward magnitudes, one and five pellets that were indicated in advance by the first stimulus. The authors found that rats released the lever more quickly on trials where they were going to receive five pellets than on trials where they were going to receive one pellet; the difference, termed RT gain, was about 45 ms. Bilateral intra-NAc injections of the D2-like receptor antagonist haloperidol were without effect on RT gain. AP5 significantly reduced RT gain but did not affect number of trials taken to complete the test session. They argued that their results showing that NAc injections of AP5 were without effect on trials to complete the test session were in good agreement with those of Kelley et al. (37) showing that, once training had taken place, NAc injections of AP5 did not affect performance. The authors concluded that NMDA but not DA D2-like receptors in NAc of well-trained rats are importantly involved in guiding the speed of instrumental responding under the control of a predictive stimulus that signals upcoming reward magnitude.

The reaction time task of Hauber et al. (64) may assess an aspect of complex behavioral control that is added to the control of operant responding by environmental stimuli. Notwithstanding their observations, it appears from the work of A. Kelley's group that Glu NMDA receptors are necessary for acquisition but not expression of lever pressing for food. Related studies implicate mGluRs in lever press learning. Studies with DA receptor antagonists similarly have shown that DA is necessary for acquisition but not

expression. However, those studies have also shown that well-trained animals repeatedly tested under the influence of a DA receptor antagonist gradually decrease responding, showing an extinction-like decline. Well-trained animals repeatedly tested after systemic treatment with an NMDA receptor antagonist did not show an extinction-like decline. It remains to be determined whether repeated tests of well-trained animals treated with intra-NAc, BLA, or mPFC injections of AP5 will show a gradual decline in responding. Results of studies that have looked at both acquisition and expression appear to show that NMDA receptors are necessary for acquisition but not expression. AMPA/kainate receptors, on the other hand, were necessary for expression. This is a general finding from many studies as will be further seen in the following subheadings.

2.2.3. Glutamate and the Expression of Lever Pressing for Brain Stimulation Reward

Since the discovery by Olds and Milner (65) that animals could be trained to perform an operant response that produced electrical stimulation of certain brain regions, there has been extensive interest in identifying the neuroanatomical and neurochemical substrates that mediate this effect. It was thought that identification of the critical substrates would point to the brain circuits that mediated the effects of reward on behavior. Extensive research led to the conclusion that DA neurons were important for brain stimulation reward (BSR) (1) and these results contributed to the now widely held view that DA neurons play a critical role in reward-related incentive learning (3). For example, Mogenson et al. (66) implanted rats with stimulating electrodes in the ventral tegmental area (VTA) and bilateral cannulae into the NAc. Once self-stimulation rates were stable, they injected the DA D2-like receptor antagonist spiroperidol into the NAc either ipsilaterally or contralaterally to the stimulating electrode. They found that ipsilateral but not contralateral injections caused a reduction in rates of responding for BSR. The observation that contralateral injections were without effect eliminated the possibility that the drug was producing its effect by impairing the rats' ability to respond. Results supported the conclusion that VTA BSR depended on stimulation of DA receptors in the NAc.

Some studies have looked at the role of Glu in BSR. Herberg and Rose (67) microinjected Glu agents into the VTA to observe effects on BSR produced by electrodes placed rostral to the VTA in the medial forebrain bundle (MFB). AP5 was without effect. Less specific Glu receptor antagonists acting on NMDA and non-NMDA receptors produced a decrease in responding. Thus, the broad-spectrum EAA antagonists cis-2,3-piperidine dicarboxylate (cPDA), γ-D-glutamylaminomethyl sulphonic acid (GAMS), or p-chlorobenzoyl-2,3-piperazine dicarboxylic acid (pCB PxDA) reduced responding. Control injections of these agents into the VTA contralateral to the side of the electrode were without effect ruling out the possibility that reductions in response rates were related to nonspecific motor effects of the broad-spectrum Glu receptor antagonists. With electrodes in the ventral pallidum, Panagis and Kastellakis (68) found no effect of VTA injections of NMDA or AMPA on BSR thresholds. The results of Herberg and Rose (67) implicate non-NMDA Glu receptors in VTA in the expression of MFB BSR and those of Panagis and Kastellakis (68) implicate neither NMDA nor AMPA Glu receptors in the VTA in the expression of ventral pallidal BSR.

The effects of intra-VTA injections are generally consistent with results from studies investigating the effects of systemic injections of Glu agents. It was found that neither of the noncompetitive NMDA receptor antagonists dizocilpine nor ketamine impaired BSR

produced by electrical stimulation of the midlateral hypothalamus; in fact, they enhanced responding at low doses (refs. *69–71*). In a related study, Ranaldi et al. *(72)* found that dizolcipine failed to affect thresholds for lateral hypothalamic BSR; however, dizocilpine augmented the threshold-reducing effects of cocaine. Besides antagonizing NMDA receptor function, dizocilpine and ketamine have been shown to activate A10 DA neurons *(73)* or to increase the metabolism of DA in several brain areas *(74,75)*: these DA effects of dizocilpine and ketamine may account for their ability to augment BSR reward. M. Olds *(71)* tested this hypothesis by evaluating the effects of the DA receptor antagonists SCH 23390 or halpoeridol on the BSR-enhancing effects of dizocilpine; both DA agents blocked the effect. Thus, NMDA antagonists fail to impair well-established lever press responding for BSR, suggesting that NMDA receptors are not involved in the expression of responding. The agents tested also produced an increase in DA neurotransmission; this increase can augment BSR reward.

Recall that Herberg and Rose *(70)* found no impairment of BSR with dizocilpine or ketamine. On the other hand, the broad-spectrum EAA receptor antagonist kynurenic acid suppressed responding maintained by BSR, elevated threshold current intensity for producing BSR, and blocked the enhancement of BSR responding produced by morphine *(70,76)*. Herberg and Rose *(67)* concluded that, "... the NMDA receptor is unlikely to play an essential role in maintaining self-stimulation in the *fully trained* rat" (italics added). Coupled with the findings reviewed in Subheadings 2.2.1 and 2.2.2 on acquisition and expression of operant responding for food, results point to a role for NMDA receptors in the establishment but not the early expression of reward-related learning with non-NMDA AMPA and kainate receptors perhaps being important for expression.

2.2.4. Glutamate and the Acquisition of Lever Pressing to Self-Administer Drugs

DA has been found to play a critical role in the acquisition of self-administration behavior. Early studies showed that animals learned to perform an operant response when an iv injection of amphetamine *(77)* or cocaine *(78)* was made contingent on that response. Since then, many DA agents have been found to be self-administered including apomorphine *(79)*, the D2-like receptor agonist bromocriptine *(80)*, and the D1-like receptor agonists SKF 82958 and SKF 77434 *(81)* and SKF 81297 *(82)* in rats or monkeys. Results suggest that DA receptor stimulation is rewarding.

Carlezon and Wise *(83)* reported that rats would self-administer the NMDA receptor antagonists PCP, dizocilpine, or (±)-CPP directly into the NAc shell or mPFC; injections into the NAc core were not effective. The rewarding effects of these agents were not blocked by coinfusion of the DA D2 receptor antagonist sulpiride. Results suggested that the NMDA receptor-antagonists injected into the NAc shell or mPFC produced reward independent of activation of DA D2 receptors. It is difficult to reconcile these findings with those of A. Kelley and colleagues (*see* Subheading 2.2.1.) showing that blockade of NMDA or DA D1-like receptors in the NAc impaired acquisition of lever pressing for food. Besides the reinforcing stimulus used, some differences between the studies are that Carlezon and Wise *(83)* made multiple response-contingent injections of a smaller volume and a different drug from the one used by A. Kelley and her coworkers; Carleson and Wise *(83)* also tested a D2-, not a D1-like DA receptor antagonist.

Mice were found to learn to choose the correct arm of a Y-maze to receive intra-VTA injections of the competitive NMDA receptor antagonist D(-)-2-amino-7-phosphonohep-tanoic acid (AP7) or the AMPA/kainate receptor antagonist DNQX *(84)*. This response

was dependent on DA D2 receptors as pretreatment with sulpiride in well-trained animals led to rapid extinction of the response. These results are consistent with the critical role of DA in reward-related learning. They suggest that normally, Glu afferents to the VTA, acting on either NMDA or AMPA receptors, inhibit DA cell firing, probably through a γ-aminobutyric acid (GABA) interneuron; Glu receptor blockade decreases this GABAergic inhibition, increasing the activity of DA neurons and producing reward. It is difficult to reconcile these findings with the report of Herberg and Rose *(67)* that broad-spectrum EAA receptor antagonists injected into the VTA decreased MFB BSR. Perhaps the use of more selective drugs in the more recent experiments allows for identification of the regionally specific function of different ionotropic Glu receptor subtypes.

The acquisition of cocaine self-administration was impaired by dizocipline. Schenk et al. *(85,86)* observed that rats treated with dizocilpine pressed both the active and inactive lever over training days. When dizocilpine was discontinued, acquisition proceeded in a manner similar to that observed in naïve rats showing that the experience with cocaine during training with dizocilpine did not lead to learning about the cocaine-producing lever.

In summary, acquisition studies show that animals will learn operant responses rewarded by intra-NAc shell, PFC, or VTA injections of NMDA receptor antagonists. Rewarding effects of these agents in the NAc shell or PFC were not blocked by local injection of the DA D2-like receptor antagonist sulpiride but VTA reward was blocked by systemic sulpiride. The latter finding is consistent with a role for VTA DA neurons in reward. Systemic injections of dizocilpine impaired acquisition of self-administration for cocaine. This finding is consistent with observations of impaired lever press acquisition for food in animals treated with NMDA receptor antagonists (Subheading 2.2.1). We know of no reports of acquisition of self-administration for intravenous injections of Glu receptor antagonists.

1.2.5. Glutamate and the Expression of Lever Pressing to Self-Administer Drugs

There are two sorts of experiments in this category. The first involves the use of a substitution procedure. Animals are initially trained to self-administer a stimulant drug, such as cocaine, and then other drugs are substituted for the cocaine to see if they will maintain responding. Because the lever press response is already well trained before the drug substitution takes place, these experiments are classified as tests of expression rather than acquisition. The second type of experiment involved assessing the effects of agents on ongoing responding for a self-administered drug. DA receptor-blocking drugs reduce the rewarding effects of self-administered stimulants. Both D1-like *(87)* and D2-like receptor blockers were effective *(88)*. In recent years it has been discovered that the rewarding effects of many substances including amphetamine, cocaine, heroin, morphine, nicotine, alcohol, and cannabinoids depend on intact function of the VTA-NAc DA system (for a review; *see* ref. *13*).

The NMDA receptor antagonists dizocilpine or PCP were self-administered in a dose-dependent manner by monkeys using the substitution procedure *(89–91)*. If monkeys were trained to self-administer cocaine and then switched directly to dizocilpine, they did not self-administer the substituted drug. When they were trained to self-administer cocaine and then switched to the noncompetitive NMDA receptor antagonist PCP, they self-administered PCP and then when dizocilpine was substituted, self-administration was seen. Thus, in well-trained monkeys, self-administration of NMDA receptor antagonists is seen under some circumstances. As the drugs were administered systemically, it

is not possible to determine where they were acting in the brain. The results of acquisition of self-administration studies using central injections (Subheading 2.2.4) provide some clues.

In other studies using the substitution procedure, mGluR5 null mutant mice failed to self-administer cocaine when it was substituted for food *(48)*. In the same study, wildtype mice treated with the mGluR5 antagonist MPEP showed reduced self-administration of cocaine. Results implicated mGluR5 in cocaine reward.

Turning to experiments involving assessment of the effects of agents on ongoing responding for a self-administered drug, injections of the NMDA receptor agonist 1-aminocyclobutane-*cis*-1,3-dicarboxylic acid (*cis*-ACDA) or AMPA into NAc decreased responding for self-administered cocaine, indicative of increased reward. This study failed to observe any effect of dizocilpine, AP5, or the AMPA/kainate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) injected into NAc *(92)*. However, studies from Koob's lab with well-trained rats showed that injections of AP5 into the NAc produced an increase in cocaine (but not heroin) self-administration, indicative of a decrease in cocaine reward *(93)*. Related studies showed that NAc injections of AP5 decreased ethanol reward *(94)*. When AP5 or CNQX was injected into the BLA, they failed to affect cocaine reward *(95)*. Results indicate the involvement of NMDA and AMPA or kainate receptors in NAc in cocaine and ethanol but not heroin reward.

Systemic administration of Glu NMDA receptor antagonists increased reward produced by cocaine. In Subheading 2.2.3 on the effects of Glu drugs on the expression of BSR, it was noted that dizocilpine or ketamine appeared to enhance reward *(70,72)*. Similar observations have been made in self-administration studies. Thus, Ranaldi et al. *(96)* showed that systemic injections of dizocilpine increased cocaine reward. Using a self-administration procedure, these authors assessed the maximum FR (breaking point) that a particular concentration of cocaine would support and found that the maximum increased with cocaine concentration. In previous studies, Roberts et al. *(97)* had shown that a DA receptor antagonist decreased breaking points. Ranaldi et al. *(96)* showed that dizocilpine increased breaking points. These results suggest that systemic administration of an NMDA receptor antagonist augmented reward produced by cocaine. Dizocilpine or (+)-HA966 decreased rates of cocaine self-administration in rats *(56,57)*. As increases in dose of cocaine per infusion also led to a decrease in rate, results support the findings of Ranaldi et al. *(96)* suggesting that systemically administered NMDA receptor antagonists augment cocaine reward. On the other hand, Schenk et al. *(85,86)* showed that dizocilpine failed to shift the dose–response curve for cocaine self-administration but it impaired discriminated responding on the cocaine-producing lever, leading to increased responding on the inactive lever.

The findings that the NMDA receptor antagonist dizocilpine augmented cocaine self-administration in most studies can be interpreted in the same manner as the findings reviewed in Subheading 2.2.3 showing a similar effect of this agent on established responding for BSR. Thus, the finding that NMDA receptor antagonists failed to decrease cocaine reward suggests that the expression of responding for cocaine does not require NMDA receptors. The finding that dizocilpine increases cocaine reward is consistent with the ability of this agent to increase activity in VTA-NAc DA neurons *(73)* and its ability to increase the metabolism of DA in several brain areas *(74,75)*.

The AMPA/kainate receptor-antagonist DNQX was found to decrease lever pressing for self-administered cocaine and the same dose decreased lever pressing for food

(*see* Subheading 2.2.2.). Pierce et al. *(57)* also had found that dizocilpine decreased responding for cocaine but had no effect on responding for food. The finding that DNQX similarly decreased responding for both types of reinforcer led Pierce et al. *(57)* to suggest that the effect of DNQX could be attributed to nonspecific motor changes. Alternatively, it could be that NMDA receptors are involved in the expression of lever press responding for cocaine but not food, whereas AMPA or kainate receptors are involved in the expression of both.

These studies can be summarized as follows. Glu NMDA receptor antagonists were self-administered when they were substituted for cocaine. Consistent with these observations, reward produced by self-administered cocaine was augmented by injection of the NMDA receptor antagonists dizocilpine or ketamine in some studies. However, conflicting results showed that systemic dizocilpine failed to shift the self-administered cocaine dose–response curve. Central injection studies showed that intra-NAc injections of an NMDA agonist or AMPA itself increased cocaine reward and AP5 decreased cocaine or ethanol but not heroin reward. These findings might suggest that the apparent rewarding effects of Glu NMDA receptor antagonists or the augmentation of cocaine reward reported following systemically administered NMDA receptor antagonists is not the result of an action of the antagonists in NAc. However, acquisition studies (Subheading 2.2.4.) showing reward based on intra-NAc infusions of NMDA receptor antagonists seem to contradict this conclusion. The finding that intra-NAc AP5 decreased cocaine or ethanol reward is not consistent with the observations of A. Kelley and her colleagues showing that AP5 failed to block expression of responding for food. Perhaps the use of different reinforcers in the studies from Koob's lab (cocaine) vs those from Kelley's lab (food) accounts for the difference. This hypothesis is supported by the findings of Pierce et al. *(57)* showing that dizocilpine decreased responding for cocaine but not for food. Finally, there is evidence implicating AMPA, kainate, and mGluR5 receptors in the expression of lever pressing for stimulant self-administration.

2.2.6. Glutamate and the Resumption (Relapse) of Lever Pressing to Self-Administer Drugs

Animals that have been trained to self-administer a drug (e.g., cocaine) eventually will cease to respond if the drug is no longer available, showing extinction. The presentation of environmental stimuli that have previously been paired with the rewarding drug or exposure to the drug stimulus itself can produce resumption (relapse) of operant responding for the rewarding drug *(98)*. Injection of DA directly into the NAc reinstated responding on a manipulandum that previously produced cocaine self-administration and this effect was blocked by NAc injection of the DA receptor-blocker fluphenazine *(99)*. Injections of SCH 23390 but not the D2-like DA receptor antagonist raclopride into the BLA blocked the ability of drug-paired stimuli to reinstate responding for cocaine *(95)*. Results implicated NAc and BLA DA in resumption of lever pressing for stimulant self-administration.

Response reinstatement produced by intra-NAc injections of DA was blocked by coinjection of the DA receptor antagonist fluphenazine or the AMPA Glu receptor antagonist CNQX; the NMDA receptor antagonist (±)-CPP was ineffective. The same was found for reinstatement produced by systemic cocaine except that NAc fluphenazine did not block the effect. This result suggested that response reinstatement produced by systemic cocaine did not depend on the action of cocaine on DA neurons in NAc *(99)*. These researchers also showed that NAc injections of the NMDA receptor-agonist *cis*-ACDA

or AMPA itself reinstated responding; the effect of AMPA was blocked by CNQX but not by fluphenazine *(92,99)*.

In rats with excitotoxic lesions of the BLA, cocaine-associated cues were ineffective in the reinstatement of extinguished lever pressing previously rewarded with cocaine but now rewarded only with a conditioned stimulus that had been paired with cocaine *(100)*. See et al. *(95)* examined the effects of AP5 or CNQX injected into the BLA in the reinstatement paradigm and observed no effect. Results implicate the BLA but not ionotropic Glu receptors in cue-induced response reinstatement.

Dizocilpine dose-dependently reinstated responding for cocaine in rats after an extinction period of 1–3 wk *(101,102)*. Dizocilpine is known to activate DA neurons and to increase regional DA concentrations *(73,75)* and treatments that increase DA are known to effectively reinstate responding for cocaine *(98)*. Thus, the observation that the NMDA receptor antagonist dizocilpine reinstates cocaine responding is consistent with many previous findings. Furthermore, these results suggest that NMDA receptor stimulation is not necessary for reinstatement. This conclusion is supported by the results of Bespalov et al. *(102)*. They showed that the NMDA receptor antagonists 3-(2-carboxypiperazin-4-yl)-1-propenyl-phosphonic acid (D-CPPene) or memantine had no effect on the reinstatement of responding produced by cocaine.

In summary, systemic cocaine, intra-NAc DA, or exposure to drug-paired cues reinstated previously extinguished responding for cocaine self-administration. The effects of NAc DA or drug-paired cues were blocked by DA or AMPA receptor antagonism but not by systemic or intra-NAc NMDA receptor antagonism. Intra-NAc NMDA or AMPA agonists produced response reinstatement; dizocilpine also produced reinstatement but its effect was probably mediated by DA. The resumption of responding for stimulant self-administration elicited by drug-paired cues was blocked by excitotoxic lesions of the BLA but not by NMDA or AMPA antagonists in BLA.

2.2.7. Glutamate and the Acquisition of Lever Pressing for Conditioned Reward

A stimulus that is repeatedly paired with a primary rewarding stimulus (e.g., food), acquires the ability to act as a reinforcing or rewarding stimulus in its own right; such a stimulus is termed a conditioned reward. Animals will learn an operant response, such as lever pressing when a conditioned rewarding stimulus is made contingent on that response. Many studies have shown that treatment with agents such as amphetamine that augment DA neurotransmission specifically enhance responding for conditioned reward and DA receptor antagonists block learning with conditioned reward. Similar enhancing effects have been reported following intra-NAc injections of amphetamine, DA, and a number of DA agonists (for a review, *see* ref. *22*).

The enhancement of responding for conditioned reward produced by intra-NAc injections of amphetamine was blocked by coinjection of AP5 or CNQX *(35,103)*. CNQX, when injected alone, also decreased the conditioned reward effect itself *(35)*. Coinjection of the Glu receptor agonists NMDA, AMPA, or quisqualate impaired the amphetamine-produced enhancement of responding for conditioned reward. NMDA alone also decreased responding on the conditioned reward lever *(35)*. Results implicate NAc Glu acting at NMDA, AMPA or kainate receptors in the effects of amphetamine on responding for conditioned reward. They suggest that there is an optimal level of receptor stimulation for the acquisition of responding: either increases to levels above or decreases to levels below that putative optimum impair learning.

BLA or ventral subicular lesions decreased responding for conditioned reward *(104–106)*. BLA lesions did not affect the ability to discriminate *(104)*. Whereas BLA lesions did not block the enhancement of responding produced by NAc amphetamine, ventral subiculur lesions did *(105)*. Microinjection of CNQX into BLA or ventral subiculum decreased responding for conditioned reward, but injections of CNQX into BLA also increased responding on a second manipulandum that produced no programmed consequences *(107)*. These results implicate BLA AMPA/kainate receptors in the control of behavior by discriminative stimuli and ventral subiculur AMPA/kainate receptors in reward efficacy.

2.3. Glutamate and Appetitive Learning: Place-Conditioning Tasks

The previous sections focused on the acquisition and expression of lever press responses rewarded by several different types of stimuli (i.e., food, BSR, psychomotor stimulants, or conditioned rewards). It is also possible to assess the rewarding qualities of stimuli by pairing them with a particular environment or place and then assessing the animals' response to that place. These types of studies can use the conditioned place preference paradigm, where the animal chooses between alternatives with differential conditioning histories, or the conditioned activity paradigm, where animals' locomotor activity is observed in an environment previously paired with a rewarding stimulus. Both types of paradigms have been used to assess the role of DA and Glu in reward-related learning.

2.3.1. Glutamate and the Acquisition of a Conditioned Place Preference

The conditioned place prefences (CPP) procedure usually involves the pairing of one chamber of a two- or three-chambered apparatus with a rewarding stimulus (e.g., cocaine or amphetamine). One or more features such as floor texture, wall design, or odor, as well as their location in space, normally distinguish the chambers. In the test, animals are given access to all chambers and the amount of time spent in each is measured. If an animal spends more time in the environment previously paired with the rewarding stimulus, a CPP is said to have occurred.

Many data implicate DA in CPP learning. Thus, DA agonists produce a CPP that is blocked by cotreatment during conditioning with DA receptor antagonists *(108)*. The rewarding effects of a number of agents including opiates also appear to depend on DA *(109)*. Central-injection studies have shown the NAc to be an important region for producing CPP effects *(110,111)*. CPP has been seen with both D1-like receptor agonists *(112)* and D2-like receptor agonists *(113)*. For a thorough review, *see* Tzchentke *(114)*.

The role of Glu in the acquisition of CPP can be considered in studies that evaluate the possible rewarding properties of Glu agents themselves or in studies that evaluate the effects of Glu agents on reward produced by other agents.

2.3.1.1. ACQUISITION OF CONDITIONED PLACE PREFERENCE WITH GLUTAMATERGIC AGENTS

The competitive NMDA receptor antagonist DL-(E)-2-amino-4-methyl-5-phosphono-3-pentonoic acid (CPG 37849) produced a CPP. Excitotoxic lesions of the infralimbic, prelimbic, or anterior cingulate regions of the mPFC blocked CPP produced by CPG 37849. A CPP based on systemic cocaine or morphine but not amphetamine was also blocked by lesions to one of these subregions of the mPFC *(115)*. These results implicated these regions of the mPFC in reward produced by the NMDA receptor antagonist CPG 37849.

PCP produced a dose-dependent conditioned place *aversion* in a number of studies *(116–119)*. This effect was blocked by the DA D1-like receptor antagonist SCH 23390 *(118)* and by the serotonin 5-HT3 receptor-antagonists ICS 205-930 or MDL 72222, implicating these monoamines in this action of PCP.

Dizocilpine produced a CPP in many studies *(120–126)*; the one exception, Tzschentke and Schmidt *(127)* tested only one dose. CPP has been reported with CPG 37849, as noted above, and its (R)-enantiomer CPG 40166 *(115,122,125,127)* and with the Glu release inhibitor riluzole *(128)*. 1-Aminocyclopropanecarboxylic acid (ACPC), a partial agonist at the strychnine-insensitive glycine site, that acts as a functional NMDA receptor-antagonist, failed to produce a CPP *(125,129)*. The low-affinity noncompetitive NMDA receptor antagonist memantine did not produce a CPP *(130)*.

We know of only one study that has evaluated the role of AMPA receptors in the acquisition of CPP with Glu agents. The selective AMPA receptor antagonist 1-(4-aminopheyl)-4-methyl-7,8-methylenedioxy-5H-2,3-benzodiazepine (GYKI 52466) did not produce a CPP *(131)*.

The nonspecific Glu receptor antagonist kynurenic acid did not produce a CPP *(76)*. Similarly, inhibitors of the enzyme N-acetylated-α-linked-acidic dipeptidase (NAAL-ADase), which hydrolyzes the dipeptide N-acetyl-aspartyl-glutamate (NAAG) to n-acetylaspartate and Glu, failed to produce a CPP. Thus, no CPP effect was seen with 2-(phosphonomethyl)pentanedioic acid (2-PMPA) or GPI 5693 *(132)*.

Papp et al. *(125)* suggested that DA may mediate the CPPs produced by dizocilpine, CPG 37849, and CPG 40166. These agents increase activity of DA neurons of the VTA-NAc system *(73–75)* and Papp et al. *(125)* cite preliminary results showing that the DA receptor antagonist haloperidol blocked the CPP produced by dizocilpine or the CPG compounds. They point out that glycine receptor antagonists like ACPC, a functional NMDA receptor antagonist that failed to produce a CPP, do not modulate the spontaneous activity of DA neurons. Thus, DA may mediate the CPPs produced by dizocilpine, CPG 37849, and CPG 40166. As these agents also block NMDA receptors, results suggest that the acquisition of a CPP to these agents can take place when NMDA receptors are blocked.

2.3.1.2. Effects of Glutamatergic Agents on Acquisition of CPP With Other Agents

The CPP produced by methamphetamine in mice was blocked by dizocipline *(133)* but a similar experiment in rats with amphetamine showed no effect *(121)*. However, in rats the Glu release inhibitor riluzole blocked the CPP produced by amphetamine *(128)* as did ACPC, a functional NMDA receptor antagonist *(129)*. NAc injections of the AMPA/kainate receptor antagonist DNQX blocked the CPP produced by amphetamine in rats *(134)* but systemic injections of the selective AMPA receptor antagonist 2,3-dihydro-6-nitro-7-sulphamoyl-benzo(f)quinoxaline (NBQX) did not *(135)*. Taken together, results suggest that NMDA and possibly NAc kainate but not AMPA receptors are critical for the acquisition of a CPP with amphetamine.

Acquisition of a cocaine-produced CPP in mice or rats was blocked by the noncompetitive NMDA receptor antagonist dizocilpine *(136,137)* and in rats by the partial agonist at the strychnine-insensitive glycine site ACPC, a drug that acts as a functional NMDA receptor antagonist *(129)*. Cocaine CPP was also blocked by NAc injections of the AMPA/kainate receptor antagonist DNQX *(138)* but not by intracerebroventricular injections

of DNQX *(136)*. The NAALADase inhibitors 2-PMPA and GPI 5693 blocked acquisition of a CPP to cocaine *(132)*. Results implicate NMDA and possibly AMPA and kainate receptors in the acquisition of a CPP with cocaine.

The CPP produced by morphine also was sensitive to manipulations of Glu function. Dizocilpine blocked the acquisition of a morphine CPP in mice *(124)* and rats *(127,131)* as did CGP 37849 in rats *(127)*. Morphine CPP was also blocked by the low-affinity noncompetitive NMDA receptor antagonist memantine *(130)* and by ACPC *(129)*. The Glu release inhibitor riluzole or the nonspecific Glu receptor antagonist kynurenic acid blocked the acquisition of a morphine CPP *(76,128)*. The acquisition of a morphine CPP was not blocked by NAc injections of the AMPA/kainate receptor antagonist DNQX *(134)*. Results implicate NMDA receptors in the acquisition of a CPP with morphine.

Agents that reduce Glu neurotransmission also blocked the acquisition of a CPP to other rewarding drugs. Thus, ACPC, a partial agonist at the strychnine-insensitive glycine site that acts as a functional NMDA receptor antagonist, blocked the CPP produced by nicotine, nomifensine, or diazepam *(129)*. These results further implicate NMDA receptors in the acquisition of CPP to rewarding drugs.

Food produces a CPP *(139)*. Acquisition of a food-based CPP was not blocked by memantine *(130)* or by ACPC *(129)*. ACPC did not affect CPP based on sucrose, social interaction, or novelty *(129)*. Results suggest that NMDA receptors may not be necessary for the establishment of a CPP based on a number of natural rewards.

With the exception of natural rewards, results from studies investigating the role of Glu in the acquisition of CPP implicate NMDA receptors. Thus, NMDA receptor antagonists blocked CPP based on amphetamine, cocaine, morphine, and several other rewarding drugs. The effects of AMPA/kainate receptor antagonists were less consistent.

2.3.2. Glutamate and the Expression of CPP

Once conditioning to one side of a CPP apparatus has taken place, the effects of various treatments on the expression of a CPP can be assessed. It has generally been found that conditioned responses that require DA for their acquisition are transiently resistant to DA receptor antagonists during the expression phase (*see* Subheading 2.2.2.). For example, CPP based on cocaine was not blocked by SCH 23390 or sulpiride in the test phase *(136)*.

The expression of a CPP based on amphetamine was blocked by NAc injections of the AMPA/kainate receptor antagonist DNQX *(134)* or by systemic injections of the similarly acting compound CNQX *(135)*. Surprisingly, systemic injections of the selective AMPA receptor antagonist NBQX did not block expression *(140)* but the similarly selective compound GYKI 52466 did *(131)*. L-701,324, an antagonist at the strychnine-insensitive glycine site, blocked expression of CPP based on amphetamine. These observations led Mead and Stephens *(135)* to argue that the effects of CNQX could be attributed to its action at the glycine site of the NMDA receptor. The observation of Papp et al. *(129)* that ACPC, a partial agonist at the strychnine-insensitive glycine site, blocked the expression of a CPP based on amphetamine is consistent with this suggestion. Bespalov *(141)* showed that the NMDA receptor antagonist (±)-CPP blocked expression of a CPP based on amphetamine. Results implicate NMDA receptors and possibly AMPA/kainate receptors in the expression of a CPP based on amphetamine.

NAc or icv injections of DNQX blocked expression of a CPP based on cocaine *(136,138)*. Dizocilpine had no effect *(136)* but ACPC produced a block *(129)*. The

NAALADase inhibitors 2-PMPA and GPI 5693 blocked expression of a CPP based on cocaine *(132)*. Results implicate AMPA/kainate receptors and possibly NMDA receptors in the expression of a CPP based on cocaine.

The expression of a CPP based on morphine was blocked by NAc injections of DNQX *(134)*. Intra-NAc or VTA injections of the NMDA receptor antagonist 2R,4R,5S-2-amino-4,5-(1,2-cyclohexyl)-7-phospoonoheptanoic acid (NPC 17742) also blocked the effect *(142)*. Systemic injections of dizocilpine, memantine, NPC 17742, GYKI 52466, or kynurenic acid blocked the expression of morphine CPP *(76,130,131,142,143)* but ACPC was without effect *(129)*. Results implicate both NMDA and AMPA/kainate receptors in the expression of a CPP based on morphine.

The expression of a CPP based on food was not affected by memantine, ACPC, 2-PMPA, or GPI 5693 *(129,130,132,143)*. Memantine blocked the expression of a CPP based on sexual interaction *(143)*. ACPC did not block the expression of a CPP based on diazepam or nicotine but did block the CPP based on nomifensine *(129)*. Results suggest that the expression of CPP based on food may be independent of Glu NMDA receptors.

2.3.3. Glutamate and the Acquisition of Conditioned Activity

When injections of a psychostimulant drug are repeatedly paired with a particular environment, that environment will acquire the ability to elicit enhanced locomotor responses in the future when the animal is placed there in a drug-free state. This effect has been observed following drug-environment pairings with amphetamine *(144)* or cocaine *(145)*. D1- or D2-like DA receptor agonists *(146,147)* also produced conditioned activity. D1- but not D2-like DA receptor antagonists blocked the establishment of conditioned activity based on amphetamine or cocaine *(147–150)*. Thus, both D1- and D2-like receptors appear to play a role in the establishment of conditioned activity.

Several studies have investigated the effects of Glu receptor antagonists on the establishment of conditioned activity. Thus, coinjections of the NMDA receptor antagonist dizocilpine with amphetamine or cocaine during conditioning blocked the conditioned activity effect; the doses of dizocilpine that blocked the effect did not affect unconditioned locomotion stimulated by amphetamine or cocaine ruling out a nonspecific motor effect *(150–152)*. Intracerebroventricular injections of the NMDA receptor antagonist (±)-CPP blocked acquisition of conditioned activity based on cocaine *(153)*. Icv injections of the AMPA/kainate receptor antagonist DNQX during conditioning sessions also resulted in a loss of the conditioned activity effect *(150)*. Results implicate NMDA, AMPA, and kainate receptors in the acquisition of conditioned activity.

2.3.4. Glutamate and the Expression of Conditioned Activity

Expression is tested after pairing sessions have taken place. When the minimal effective dose for blocking acquisition of the effect was identified and used in the test, it was found that the D2-like DA receptor antagonist pimozide failed to block expression of conditioned activity based on amphetamine or cocaine *(154,155)*. Similar results have been reported for haloperidol *(156)*. SCH 23390 decreased activity levels in amphetamine-conditioned and unpaired groups when given in the test, but it failed to block the expression of conditioned activity based on cocaine *(150)*. When considered in conjunction with the effects of DA receptor antagonists reviewed in Subheading 2.3.4, results suggest that DA acting at both receptor families plays a more important role in the establishment than expression of conditioned activity.

NAc injections of the NMDA Glu receptor-antagonist (±)-CPP dose-dependently blocked expression of conditioned activity based on systemic amphetamine or morphine; similar injections into the dorsal striatum were without effect *(157)*. Systemic injections similarly decreased expression of conditioned activity based on systemic cocaine. Thus, dizocilpine, memantine, D-CPPene, the glycine site antagonist 5-nitro-6,7-dichloro-1,4-dihydro-2,3-quinoxalinedione (ACEA-1021), or the polyamine site antagonist eloprodil decreased the expression of conditioned activity; memantine, ACEA-1021, and eloprodil did this at doses that did not affect spontaneous activity *(158)*. In another study, dizocilpine or icv (±)-CPP failed to block expression of conditioned activity based on cocaine *(150,153)*. Results were conflicting. Some implicated NMDA Glu receptors, possibly in NAc, in the expression of conditioned activity and some results were negative.

In mice conditioned with amphetamine, the AMPA receptor antagonist NBQX blocked expression of the conditioned activity effect at a dose that did not affect spontaneous locomotor activity *(140)*. DNQX similarly blocked the expression of conditioned activity based on cocaine conditioning *(150)*. In rats conditioned with cocaine, the AMPA receptor antagonist GYK 52466 blocked expression of the conditioned activity effect *(159)*. This latter study used intracerebral microdialysis of NAc to show that conditioned stimuli associated with cocaine increased Glu release. Results implicate AMPA and kainate, along with NMDA receptors in the expression of conditioned activity.

3. MECHANISMS OF LEARNING

In recent years, the molecular mechanisms of learning have been extensively studied. This work is exemplified by investigations of the sea slug *Aplysia (160)*. However, numerous other species have been studied. Thus, somewhat similar mechanisms have been identified in *Caenorhabditis elegans (161)*, *Drosophila (162)*, honey bee *(163)*, chick *(164)*, and rat *(160,165,166)*. There appears to be a high level of conservation of the mechanisms for producing learning and memory across phylogeny. This apparent conservation extends to the putative mechanism underlying learning produced by rewarding stimuli.

3.1. Role of Glutamate and Dopamine in the Striatum and NAc

Beninger *(3)* first proposed a synaptically based mechanism for reward-related learning. He proposed a heterosynaptic facilitation model involving the ability of DA afferents, acting at D1-like receptors, to modulate coterminating cholinergic afferents on medium spiny straital neurons; at the time there was good evidence for such an interaction between DA and cholinergic synapses but little evidence for a DA–Glu interaction. In the following decade, evidence for a DA–Glu interaction accumulated, leading Wickens *(21,167,168)* and his coworkers *(6,169)* to propose that DA-mediated reward-related learning occurred as a result of the modulation by DA of Glu synapses made by cortical afferents on the spines of medium spiny striatal neurons. There is now extensive electrophysiological and neurochemical evidence supporting this model *(170–174)*.

The model fits into the following context (cf. ref. *175*). Most of the neurons in the striatum and the NAc are of the medium spiny type. These neurons use GABA as their principal neurotransmitter and are the principal projection neurons of the striatum *(176)*. The spines of these neurons receive Glu inputs from cortical neurons and DA inputs from mesencephalic neurons *(177)*. Among other things, cortical inputs carry information

about the perception of stimuli in the environment and the output neurons of the striatum and NAc influence motor action. DA, by modifying the strength of Glu synapses in the striatum and NAc, would be able to change the behavioral impact of associated environmental stimuli that activate those synapses. One important feature of the model is that DA, although released at multiple synapses when a rewarding stimulus is encountered, would only act to strengthen Glu synapses that were recently active when stimuli associated with reward were present; that is, at Glu synapses that are in a state of readiness (178). As outlined in the next Subheading, the molecular events underlying DA-mediated learning are beginning to yield to the efforts of researchers.

3.2. Role of Signaling Molecules in Reward-Related Learning

Space does not permit an extensive review of the molecular signaling cascades that are thought to mediate the modulating influence of DA on Glu synapses and the reader is referred to several recent reviews (22,23,179,180). The series of events might include the following: When environmental stimuli are encountered, a subset of allo- and neocortical cells is activated and their corresponding synapses in the striatum and NAc release Glu. This event leads to stimulation of NMDA receptors and an increase in calcium concentrations ($[Ca^{2+}]$) in the dendritic spines that receive these synapses. Wickens (21) proposed that this event might represent the state of readiness proposed by Miller (178). Increased spine $[Ca^{2+}]$ leads to activation of enzymes including Ca^{2+}-dependent protein kinase (PKC) and Ca^{2+}- and calmodulin-dependent protein kinases (CaMKs); these enzymes phosphorylate a variety of proteins including, for example, AMPA receptors, altering their open time, and are known to be necessary for some of the molecular signals produced by stimulation of D1-like DA receptors (181,182). In the absence of a DA input in close temporal contiguity with the Glu input that establishes this state of readiness, the enzyme protein phosphatase I (PP I) will dephosphorylate recently phosphorylated proteins and undo the putative state of readiness.

When reward occurs and DA is released, stimulation of D1-like receptors will lead to activation of adenylyl cyclase and stimulation of cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA). PKA phosphorylates DA- and cAMP-regulated phosphoprotein (DARPP-32), which, in turn, inhibits PP I. As a result, the newly phophorylated proteins can endure within the synaptic spine; for example, recently phosphorylated AMPA receptors would remain phosphorylated. In addition, activation of PKA leads to activation of cAMP-response-element-binding-protein (CREB), a transcription factor involved in gene expression. CREB activation requires not only PKA but also stimulation of NMDA receptors and increases in $[Ca^{2+}]$ (181,182); this makes it an excellent candidate for mediating temporally contiguous activation of DA and Glu receptors on synaptic spines of medium spiny neurons.

These are only a few of the many molecular events that occur upon stimulation of DA or Glu receptors. Another class of enzymes is the mitogen-activated protein kinase (MAPK) family. These include extracellular signal-regulated kinase (ERK), which has been implicated in learning and memory (183); ERK mediates the ability of PKC to phosphorylate CREB. DA directly activates the p38 MAPK in a PKA-dependent manner and it activates the transcription factors CREB and Elk-1 (184). c-Jun-*N*-terminal kinase (JNK) is another MAPK that phosphorylates activating transcription factor 2 (ATF-2), a CREB family member (185). The ability of amphetamine to activate MAPKs has been

found to depend on mGluRs *(186)*. These kinases also might play a role in reward-related learning. Relevant studies are reviewed in the next section.

3.3. Pka and Reward-Related Learning

3.3.1. PKA and the Acquisition of Approach Responses

Appetitive conditioning is dependent on a corticostriatal circuit involving the BLA *(136)* and PKA manipulations in the BLA affect the acquisition of approach behavior. Jentsch et al. *(187)* infused cholera toxin (CTX), the PKA inhibitor adenosine 3′,5′-cyclic phosphorothioate-Rp (Rp-cAMPS), or the PKA activator adenosine 3′,5′-cyclic phosphorothioate-Sp (Sp-cAMPS) into the BLA and assessed acquisition of approach responses (nose pokes into a food tray) to a conditioned stimulus signaling food. G proteins of the G_s family are positively coupled to the cAMP-PKA pathway. CTX, binding to G_s, prolongs the activation of G_s proteins effectively upregulating the cAMP-PKA pathway. BLA CTX increased approaches to the food tray during the conditioned stimulus. The same was true for lower doses of Sp-cAMPS infused either immediately before or after the training sessions, but a higher dose of Sp-cAMPS decreased food tray approach. Pretraining BLA infusions of Rp-cAMPS decreased approach responses. Baldwin et al. *(33)* also analyzed nose pokes into the food tray in the context of a lever pressing task. Consistent with Jentsch et al. *(187)*, they found that mPFC Rp-cAMPS impaired the acquisition of nose pokes. In an identical task, Baldwin et al. *(188)* found that NAc infusions of the broad serine/threonine kinase inhibitor H7, Rp-cAMPS, or Sp-cAMPS impaired acquisition of approach responses.

Results with agents that reduce PKA activity in the NAc, BLA, or mPFC were consistent in showing impairment in the acquisition of approach responding during an appetitive conditioned stimulus. Agents that augmented PKA activity in the BLA augmented learning at low doses but impaired it at higher doses and these agents in NAc impaired learning. Results implicate NAc, BLA, and mPFC PKA in the acquisition of approach responses during conditioned stimulus presentation.

3.3.2. PKA and the Acquisition of Lever Pressing for Food

PKA may be necessary for the acquisition of lever pressing for food. Baldwin et al. *(188)* trained rats to lever press for food over 10 d on an FR 1 schedule. Drug manipulations were introduced on days 1–4 to study the role of PKA in acquisition. Immediate posttraining NAc infusion of H7 or immediate pretraining infusion of the PKA inhibitor Rp-cAMPS dose-dependently impaired acquisition of lever pressing for food. Smaller impairments were produced by infusion of Rp-cAMPS immediately or 1 hr after training sessions. Acquisition also was impaired by NAc infusion of the PKA activator Sp-cAMPS. This finding suggested that reward-related learning occurred at an optimal window of activation for PKA and that either lower or higher levels of activation resulted in impairment. A. Kelley's group also showed that infusion of Rp-cAMPS into the mPFC 5 min before training impaired learning *(188)*.

In summary, inhibition of PKA in NAc or mPFC impaired the acquisition of lever press responding for food. Stimulation of PKA in NAc also impaired acquisition.

3.3.3. PKA and the Expression of Lever Pressing for Food

To assess the role of PKA in the expression of lever pressing for food, Baldwin et al. *(188)* injected rats on test day 10 of training for lever pressing for food with Rp- or

Sp-cAMPS into NAc. Neither drug impaired the expression of lever pressing for food. Results reported by Self et al. *(189)* appear to agree with the these findings. In a study on self-administration of cocaine, Self et al. *(189)* tested a food reward group in which rats had been trained to lever press for food pellets on an FR 1/time-out 2-min schedule. Neither Rp- nor Sp-cAMPS, injected into NAc 30 min before testing, impaired lever pressing for food on the FR 1 schedule. However, Rp-cAMPS decreased responding during time-out periods and on an inactive lever. Neither of these studies tested the effects of repeated NAc treatments with a PKA inhibitor on the expression of lever pressing for food. Results showed that established responding was resistant to PKA inhibition in NAc on the first day of drug testing.

3.3.4. PKA and the Expression of Lever Pressing for Stimulant Self-Administration

Self et al. *(190)* found that inhibition of NAc G proteins G_i and G_o with pertussis toxin (PTX) produced long-lasting (up to a month) changes in iv self-administration of cocaine or heroin. PTX produced a rightward shift in the dose–response curve for both drugs. This effect was consistent with reducing the self-administered dose of the drug, causing the animal to compensate by increasing drug intake. Thus G_i/G_o proteins may be necessary for the rewarding effects of cocaine and heroin. G_i/G_o proteins are negatively coupled to the cAMP-PKA pathway, suggesting that an upregulation of PKA may have resulted in decreased reward and hence higher responding.

Self et al. *(189)* directly studied the role of PKA in cocaine self-administration. When multiple doses of cocaine were tested, the PKA inhibitor Rp-cAMPs produced a leftward shift in the dose–response curve, consistent with an *enhancement* of reward. The opposite was found for Sp-cAMPS, suggesting that increased activation of PKA decreased reward. In addition, Rp- but not Sp-cAMPs induced relapse in cocaine seeking when injected into the NAc and enhanced cocaine-induced relapse of cocaine seeking. The effects of PKA inhibition and PKA activation resembled the effects of respectively increasing and decreasing the unit dose of cocaine per injection; this observation suggested that the levels of PKA activation varied negatively with the rewarding properties of cocaine! The finding that PKA inhibition failed to block established responding for stimulant self-administration is consistent with the finding that PKA inhibition failed to affect established lever press responding for food (Subheading 3.2.3.) but the apparent increase in reward with PKA inhibition appears to be inconsistent. The difference may be explained by the nature of the self-administration paradigm. Animals were trained to self-administer cocaine in daily sessions over a period of 10 d before drug testing. As it has been shown that this may result in long-term adaptations at the cellular level (e.g., ref. *180*), it is possible that the functional role of PKA was affected by these changes.

3.3.5. PKA and the Acquisition of Lever Pressing for Conditioned Reward

Kelley and Hollahan *(191)* paired a compound light/click stimulus with food over several days. They then injected rats with NAc CTX and evaluated the acquisition of lever pressing for the compound stimulus alone. Responding was markedly enhanced by infusion of CTX into the NAc but not into the dorsal striatum. Results suggest that enhanced coupling of G_s proteins to receptors in NAc and subsequent increased activation of PKA, which occurs when G_s-coupled receptors are stimulated by DA acting at D1-like receptors, increases the acquisition of lever press responding for conditioned reward.

3.3.6. PKA and the Acquisition of a CPP

CPP is produced by NAc injections of amphetamine during pairing sessions *(110,111)*. Beninger et al. *(192)* found that coinjections of amphetamine plus the PKA inhibitor Rp-cAMPS produced a dose-dependent blockade of the CPP effect. Rp-cAMPS or the PKA activator Sp-cAMPS alone failed to affect time spent on the paired side. Coinjections of a subthreshold dose of amphetamine plus Sp-cAMPS also failed to affect side preference. On the other hand, coinjection of a dose of amphetamine that produced a CPP on its own plus the PKA activator Sp-cAMPS during conditioning led to a loss of the CPP effect.

Cocaine CPP may also be mediated by PKA. Icv infusions of the nonselective protein kinase inhibitor H7 impaired systemic cocaine-induced CPP when infused immediately before or after each conditioning session. The PKA inhibitor H89 when given immediately after each conditioning session also impaired the cocaine CPP *(193)*.

Results with Rp-cAMPS suggested that PKA activation consequent to injections of amphetamine into NAc was necessary for the establishment of a CPP and those with icv H7 or H89 similarly suggested that PKA activation may be necessary for the acquisition of a CPP produced by cocaine. The finding that CPP acquisition based on NAc amphetamine was impaired by activation of PKA was consistent with the similar findings that acquisition of approach responses to an appetitive conditioned stimulus (Subheading 3.3.1.) or lever pressing for food (Subheading 3.3.2.) were impaired by Sp-cAMPS injected into NAc.

3.3.7. PKA and the Expression of a CPP

One study reported on the effects of H7 injected icv during testing following conditioning with systemic cocaine. There was no effect on the expression of cocaine CPP *(193)*. Although H7 is a nonspecific serine/threonine kinase inhibitor, this result is consistent with the finding that the expression of lever pressing for food (Subheading 3.3.3.) or stimulant self-administration (Subheading 3.3.4.) was not blocked by PKA inhibition.

3.3.8. PKA and the Acquisition or Expression of Conditioned Activity

Conditioned activity resulting from pairing NAc amphetamine administration with the test environment was blocked dose-dependently by coinfusion of Rp-cAMPs *(194)*. NAc infusions of Rp-cAMPs enhanced unconditioned amphetamine-induced locomotion on conditioning days showing a dissociation of the role of PKA in locomotor activity vs learning. Results of a related study showed that NAc PKA inhibition on the test day not only failed to block the expression of conditioned activity, it enhanced the effect (Beninger et al., in prep.). Results support previous findings implicating PKA in the acquisition but not the expression of reward-related learning.

3.4. PKC and Reward-Related Learning

As mentioned in Subheading 2.2.1., Ungerer and associates evaluate the effects of treatments on the acquisition of lever pressing for food by injecting drugs after training and evaluating their effect on the spontaneous improvement normally seen 24 h later. Using this approach, Stemmelin et al. *(195)* showed that mice injected icv with the PKC inhibitor GF 109203X failed to show spontaneous improvement. Results supported a role for PKC in the acquisition of lever pressing for food.

A few recent studies have implicated PKC in the acquisition of a CPP based on amphetamine, cocaine, or morphine. Intra-NAc co-infusions of the PKC inhibitor NPC 15437 before each conditioning session impaired CPP produced by NAc injections of amphetamine *(196)*. Similarly, icv injection of the PKC inhibitor chelerythine immediately after but not before pairing sessions impaired CPP produced by systemic cocaine *(193)*. No CPP was observed following injection of the PKC inhibitors NPC 15437 alone into NAc *(196)* or calphostin C alone icv *(197)*.

The opioid morhpine also has the ability to elicit a robust CPP and this effect requires intact DA transmission *(198)*. Narita et al. *(197)* found that icv infusion of the PKC inhibitor calphostin C impaired place preference produced by morphine. These authors also tested mutant mice lacking the PKCγ gene. These mice did not show morphine-produced CPP suggesting that the PKCγ isoform mediates the rewarding effects of morphine.

Paradigms other than CPP have also been investigated. One recent study implicated the PKCγ isoform in associative learning for drug-related cues *(199)*. These authors paired a conditioned stimulus with cocaine injection in a self-administration procedure. Subsequent presentation of the conditioned stimulus alone resulted in upregulation of PKCγ expression in NAc core and BLA, suggesting that PKCγ may play a role in expression of learning in this paradigm.

In summary, PKC inhibition in NAc blocked CPP produced by NAc amphetamine and icv PKC inhibition blocked CPP produced by cocaine or morphine. Morphine-produced CPP was also absent in mutant mice lacking the PKCγ gene. PKCγ levels were increased in the NAc core and BLA following presentation of reward-related cues. Results implicate PKC in reward-related learning.

3.5. MAPK and Reward-Related Learning

The MAPKs include three subfamilies: ERK, p38, and JNK. Some recent work has implicated MAPKs in reward-related learning. None of the ERK inhibitor PD98059, the p38 inhibitor SB23580, or the JNK inhibitor SP600125 injected alone into NAc produced a CPP *(200)*. However, MAPKs may mediate CPP produced by cocaine, amphetamine, or morphine.

Systemic administration of the ERK inhibitor SL 327 impaired cocaine-induced CPP and cocaine-stimulated locomotion in mice *(201)*. Our lab has recently performed experiments testing the effects of all three MAPK subtypes on NAc amphetamine-produced CPP. We found that the ERK inhibitor PD98059 and the p38 inhibitor SB23580 but not the JNK inhibitor SP600125 dose-dependently impaired amphetamine-produced CPP when injected into NAc 10 min before NAc amphetamine on conditioning days *(200)*. Unlike Valjent et al. *(201)*, we did not observe a decrease in amphetamine-produced locomotion during conditioning sessions. This finding showed a dissociation between the ability of NAc amphetamine to produce an increase in activity and its ability to produce a CPP.

The ERK MAPK subfamily includes ERK1 and ERK2. Most behavioral work has involved manipulations that did not discriminate between these two kinases. One recent study suggests that this approach may be an oversimplification. Mazzucchelli et al. *(202)* found that ERK1 knockout mice showed enhanced striatal ERK2 activation after an ip injection of the D1-agonist SKF 38393. Moreover, in contrast to the studies described above the ERK1 mutants showed an enhanced systemic morphine CPP. Clearly, more research is needed to better understand the separate contributions of ERK1 and ERK2 to reward-related learning.

4. CONSIDERATION OF THE EVIDENCE FOR A ROLE FOR GLUTAMATE IN REWARD-RELATED LEARNING FROM THE POINT OF VIEW OF THE MECHANISM OF DA–GLU INTERACTIONS IN LEARNING

If the acquisition of reward-related learning involves a DA–Glu interaction like that described in Subheading 3, it would be expected that NMDA receptor antagonists would impair this acquisition. Furthermore, if reward-related learning is mediated in part by a change in AMPA or kainate receptors, it might be expected that AMPA/kainate receptor antagonists would block expression of conditioned responses. As corollaries to these hypotheses, it might further be expected that NMDA receptor antagonists would have less of an effect on the expression of conditioned responses and that AMPA/kainate receptor antagonists would have less of an effect on the acquisition of conditioning. What do the data show?

Results from the data reviewed in Subheading 2 are summarized in Table 1. It is clear that NMDA receptor antagonists impaired acquisition of reward-related learning. An effect was observed in every case where data were available with one exception. The exception was that an NMDA receptor antagonist did not block acquisition of a CPP based on food; however, only two compounds have been tested and more data are needed. Results revealed that NMDA receptor antagonists impaired acquisition of responding in conditioned approach, lever press and place conditioning tasks based on a number of rewarding agents. Although limited data are available for specific brain regions, where regional specificity was examined, the NAc core, BLA, and PFC were implicated with a small number of studies implicating the NAc shell and VTA. It appears that NMDA receptors are necessary for the acquisition of reward-related learning.

Table 1 also reveals that AMPA/kainate receptors appear to be necessary for the expression of reward-related learning. With the exception of lever pressing to self-administer cocaine, where CNQX injected into the NAc or BLA had no effect, decreases in the expression of reward-related learning were observed in every case. Thus, the expression of lever pressing for food or BSR, place conditioning based on amphetamine, cocaine, or morphine, or conditioned activity based on either cocaine or amphetamine was decreased by an AMPA/kainate receptor antagonist. The NAc has been implicated. These results suggest that AMPA/kainate receptors are necessary for the expression of reward-related learning.

It was suggested that a corollary to the observation that NMDA receptors are necessary for the acquisition of reward-related learning is the hypothesis that they may not be necessary for the expression of this type of learning. As can be seen in Table 1, this was often observed. The expression of conditioned approach, lever pressing for food, BSR, or cocaine, or CPP or conditioned activity based on cocaine was not blocked by NMDA receptor antagonists in some studies. However, conflicting results (no effect or a decrease) were found in a number of cases and only a decrease was reported in others. It is interesting to note that the only column in Table 1 where conflicting data are reported within a particular paradigm and brain region is the one for expression of reward-related learning following treatment with an NMDA receptor antagonist. As discussed in Subheading 2.2.2., one explanation for the conflicting data relates to dose. In studies of the effects of DA receptor antagonists on established responding for reward, some studies have reported little initial effect whereas others have found a decrease. However, if care is taken to identify the minimum effective dose needed to block acquisition and then that

Table 1
Summary of the Effects of NMDA and AMPA/Kainate Glutamate Receptor Antagonists Injected Systemically or Regionally on the Acquisition and Expression of Responding for Reward in a Number of Paradigms

Paradigm	Region	NMDA antagonist		AMPA/kainate antag	
		Acquisition	Expression	Acquisition	Expression
Conditioned approach	Systemic/icv	Decrease	No effect	—	—
	NAc core	Decrease	No effect	No effect	—
	BLA	Decrease	No effect	—	—
	PFC	Decrease	No effect	—	—
Lever press: food	Systemic/icv	Decrease	No effect/Dec	—	Decrease
	NAc core	Decrease	No effect	—	—
	NAc shell	Decrease	No effect	—	—
	BLA	Decrease	No effect	—	—
	PFC	Decrease	No effect	—	—
Lever press: BSR	Systemic/icv	—	No effect[a]	No effect	—
	VTA	—	—	No effect	Decrease
Lever press: cocaine	Systemic/icv	Decrease	No effect[a]/Dec	—	Decrease
	NAc	—	No effect/Dec	—	No effect
	BLA	—	No effect	—	No effect
CPP: amphetamine	Systemic/icv	Decrease	Decrease	No effect[b]	Decrease
	NAc	Decrease	—	Decrease	Decrease
CPP: cocaine	Systemic/icv	Decrease	No effect/Dec	No effect	Decrease
	NAc	—	—	Decrease	Decrease
CPP: morphine	Systemic/icv	Decrease	Decrease	—	—
	NAc	—	Decrease	No effect	Decrease
CPP: food	Systemic/icv	No effect	No effect	—	—
Conditioned act to co-caine or amphetamine	Systemic/icv	Decrease	No effect/Dec	Decrease	Decrease
	NAc	—	Decrease	—	—

[a]Increase observed probably due to increased DA neurotransmission (*see* Subheading 2.2.3).
[b]AMPA-specific antagonist NBQX.
—indicates no data available.
Antag, antagonist; BLA, basolateral amygdala; BSR, brain stimulation reward; CPP, conditioned place preference; Dec, decrease; icv, intracerebroventricularly; NAc, nucleus accumbens; PFC, prefrontal cortex; VTA, ventral tegmental area.

dose is tested on the expression of previously conditioned responding, minimal effects are seen. An example of this approach can be found in the elegant studies from A. Kelley's lab. These studies repeatedly have shown that doses of DA receptor antagonists

that block acquisition of responding for food reward have little or no effect on expression. It is well-known that higher doses of DA receptor antagonists produce decreases in motor activity or catalepsy so the observation that they also decrease conditioned responding based on reward is not surprising. By analogy to the observations from studies using DA receptor antagonists, perhaps the studies that have found that NMDA receptor antagonists impair the expression of reward-related learning have used higher doses than those necessary to block acquisition. These considerations and the results summarized in Table 1 suggest the tentative conclusion that NMDA receptors play a less important role in the expression than in the acquisition of reward-related learning. However, it is possible to disrupt the expression of conditioned responses with NMDA receptor antagonists. Further studies are needed to evaluate the hypothesis that this latter effect occurs at higher doses.

A second corollary to the finding that AMPA/kainate receptors are necessary for the expression of reward-related learning is the suggestion that they are less important for acquisition. Although fewer studies have been done in this category, examination of Table 1 reveals that when the effects of AMPA/kainate receptor antagonists were evaluated on the acquisition of reward-related learning, often no effect was seen. Thus, the acquisition of conditioned approach, lever pressing for BSR, and amphetamine, cocaine, or morphine CPP was unaffected by an AMPA/kainate receptor antagonist. On the other hand, a decrease in acquisition of amphetamine or cocaine CPP was reported following NAc DNQX and icv administration of this agent blocked acquisition of conditioned activity based on cocaine. Overall, results favor the conclusion that AMPA/kainate receptors are less important for acquisition than for expression of reward-related learning, but there are sufficient contradictory findings to keep this conclusion tentative until further studies are carried out.

Researchers studying other aspects of cognition also have concluded that NMDA Glu receptors appear to play a differential role in acquisition vs expression. For example, in a recent-memory task on the radial maze, it was found that the noncompetitive NMDA receptor antagonist dizocilpine impaired acquisition; when rats were pretrained on the maze and then tested with dizocilpine, no effect was observed *(203)*. These results are consistent with those reviewed here for reward-related learning (Table 1) and suggest that the acquisition but not the expression of recent memory may similarly depend on NMDA Glu receptors.

The effects of manipulations of Glu neurotransmission have been evaluated extensively in tests of spatial learning and fear conditioning. A review of these studies is beyond the scope of the present paper. Riedel et al. *(204)* have recently reviewed this material.

5. CONCLUSIONS

Although there are conflicting results, when the data reviewed in Subheading 1 are taken together (Table 1) they appear to provide support for the DA–Glu interaction model of reward-related learning presented in Subheading 3. Subheadings 3.3.–3.5. reviewed the relatively small number of studies that have evaluated the contribution of signaling molecules that are affected by DA and/or Glu to reward-related learning. Results supported a role for signaling pathways in reward-related learning and indirectly supported the DA–Glu interaction model. It will remain the task of future studies to

continue to explore the contribution of Glu receptor subtypes, their interaction with DA, and the contribution of signaling molecules in specific brain regions to reward-related learning. Continued success at this enterprise will move us closer to the identification of more effective treatments for a range of human disorders, many reviewed in this volume, that have been linked to DA–Glu interactions in the brain.

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