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The Role of Signaling Molecules in Reward-Related Incentive Learning

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Reward-related incentive learning involves the acquisition by neutral stimuli of an enhanced ability to elicit approach and other responses. Previous studies have shown that both dopamine (DA) and glutamate (Glu) play critical roles in this type of learning. Signaling molecules are intracellular messengers that participate in the influence of transmitter-receptor events on intracellular function including transcription in the nucleus. In recent years studies have begun to implicate signaling molecules in incentive learning. Thus, inhibition of cyclic adenosine monophosphate-dependent protein kinase (PKA) in the nucleus accumbens (NAc), that is activated by DA acting at D_1-like receptors, blocks the acquisition of conditioned approach responses, lever pressing for food, conditioned place preference (CPP) based on NAc injections of amphetamine or cocaine, and conditioned activity based on NAc injections of amphetamine. Similar effects have been observed with PKA inhibition in the basolateral amygdala or medial prefrontal cortex. If animals were trained prior to testing with PKA inhibitors in NAc, no effect was seen suggesting that PKA is more important for acquisition than expression of incentive learning. Inhibition of calcium-dependent protein kinase or mitogen-activated protein kinases in NAc similarly has been shown to block the acquisition of incentive learning. Results support a model of DA-Glu synaptic interactions that form the basis of incentive learning.

Keywords: Dopamine; ERK; Glutamate; Incentive learning; JNK; p38; PKA; PKC; MAPK; Review; Reward; Signaling molecules

INTRODUCTION

The consequences of neurotransmitters binding to metabotropic receptors include synaptic modifications that form the substrate of learning and memory (Kandel, 2001). Between the neurotransmitter-receptor binding event and the eventual long-term modification of synaptic effectiveness is a complex series of intracellular molecular interactions. The molecules that mediate these interactions are termed signaling molecules because they transmit signals to the nucleus about events at the cell membrane. Within the nucleus, signaling molecules influence the transcription of genes leading to the formation of messenger ribonucleic acids (mRNAs). These mRNAs eventually are translated into proteins that form the bases of enzymatic and structural modifications that can alter the sensitivity of the cell to particular inputs in the future. Learning is a change in responsiveness to a particular stimulus as a result of prior experience with that stimulus and memory is the cellular modifications that mediate that change. Signaling molecules provide the link between stimuli that lead to synaptic actions and changes in the responsiveness of cells to those stimuli in the future.

Extensive evidence implicates the neurotransmitter dopamine (DA) in reward-related incentive learning (Beninger, 1983; Miller *et al.*, 1990; Berridge and Robinson, 1998). 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 (Wickens, 1990; Sutton and Beninger, 1999; Kelley and Berridge, 2002). Many data suggest that DA afferents interact with glutamatergic

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(Glu) afferents common to the same cell when reward-related learning occurs (see Sutton and Beninger, 1999) and Glu clearly plays an important role in this form of learning (Beninger and Gerdjikov, 2004). Results further suggest that a number of signaling molecules activated by Glu or 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 Sutton and Beninger, 1999). These signaling molecules are the topic of the present review.

Signaling molecules that have been implicated in reward-related learning include cyclic 3',5'-adenosine monophosphate (cAMP)-dependent protein kinases (PKA), calcium (Ca^{2+})-dependent protein kinases (PKC) and mitogen-activated protein kinases (MAPK). Other signaling molecules also have been implicated: viz., DA and cAMP-regulated phosphoprotein, 32 kDa (DARPP-32), Inhibitor 1 (I-1), protein phosphatase 1 (PP-1), cAMP response element-binding proteins (CREB) and Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII). The roles of each of these signaling molecules in reward-related learning are reviewed. In each case, data are considered for a number of paradigms and, furthermore, where possible, results are considered separately for acquisition vs. expression of learning. Paradigms include conditioned approach responses, lever pressing for food, stimulant self-administration, lever pressing for conditioned reward, conditioned place preference and conditioned activity. Data are most extensive for studies of PKA, and results for each paradigm are reviewed in separate sections. For PKC and MAPK, available data are more limited and for the other signaling molecules listed above, very few data are available. A brief overview of a model of signaling molecular cascades thought to underlie reward-related learning and a consideration of how well the data reviewed here fit or contradict this model follows the review.

PKA AND REWARD-RELATED LEARNING

PKA and the Acquisition of 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 (Bindra, 1974). This type of learning is manifested in the increase in approach responses to the food tray in operant chambers after food has been delivered there on a number of occasions. Studies using systemic drug administration implicate DA and Glu *N*-methyl-D-aspartate (NMDA) receptors in the acquisition and long-term maintenance of conditioned approach responses to conditioned appetitive stimuli but, once established, this conditioning may be for a time resistant to the effects of DA or Glu NMDA receptor antagonists (review: Beninger and Gerdjikov, 2004).

Appetitive approach conditioning is dependent on corticolimbic and corticostriatal circuits involving the basolateral amygdala (BLA) (Hitchcott and Phillips, 1998) and PKA manipulations in the BLA affect the acquisition of approach behavior. Jentsch *et al.* (2002) 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. CTX affects G proteins, receptor-linked proteins that couple receptors to signaling cascades within the cell. Those of the Gs family are positively coupled to the activation of PKA. CTX binds to Gs proteins, prolonging their activation and effectively up-regulating PKA. 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. Pre-training BLA infusions of Rp-cAMPS decreased approach responses. Baldwin *et al.* (2002a) also analyzed nose-pokes into the food tray in the context of a lever-pressing task. Consistent with Jentsch *et al.* (2002), they found that Rp-cAMPS injected into the medial prefrontal cortex (mPFC) impaired the acquisition of nose pokes. In an identical task, Baldwin *et al.* (2002b) 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 PKA acting in the NAc, BLA and mPFC in the acquisition of approach responses during conditioned stimulus presentation.

PKA and the Expression of Approach Responses

Once conditioning has occurred it is temporarily resistant to disruption by DA or Glu NMDA receptor antagonists (review: Beninger and Gerdjikov, 2004). The same has been observed with NAc core injections of

agents that affect PKA. Thus, Baldwin *et al.* (2002b) showed that NAc core injections of Rp-cAMPS or Sp-cAMPS over 4 days retarded the acquisition of approach responses to stimuli that signaled food; however, once responding was established, the same injections had no effect. Results show that the expression of conditioned approach responses to food is resistant to the disruptive effects of NAc core injections of a PKA inhibitor or activator.

PKA and the Acquisition of Lever Pressing for Food

The acquisition of lever pressing for food in rats is a form of appetitive instrumental learning. 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. Both DA and Glu NMDA receptors are necessary for this form of learning (review: Beninger and Gerdjikov, 2004).

PKA may be necessary for the acquisition of lever pressing for food. Baldwin *et al.* (2002b) trained rats to lever-press for food over 10 days on a fixed ratio (FR) 1 schedule. Drug manipulations were introduced on days 1-4 to study the role of PKA in acquisition. Immediate post-training NAc infusion of H7 or immediate pre-training 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 h 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. Results also showed that infusion of Rp-cAMPS into the mPFC 5 min before training impaired learning (Baldwin *et al.*, 2002b). Thus, inhibition or stimulation of PKA in NAc, or inhibition of PKA in mPFC impaired the acquisition of lever press responding for food.

PKA and the Expression of Lever Pressing for Food

Numerous studies have shown that the expression of 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). Studies also have shown that the expression of lever pressing for food is initially resistant to the effects of Glu NMDA receptor antagonists (review: Beninger and Gerdjikov, 2004).

To assess the role of PKA in the expression of lever-pressing for food, Baldwin *et al.* (2002b) 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.* (1998) appear to agree with these findings. In a study on self-administration of cocaine, Self *et al.* (1998) tested a food reward group in which rats had been trained to lever-press for food pellets on a 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.

PKA and the Expression of Lever Pressing for Stimulant Self-administration

DA receptor blocking drugs reduce the rewarding effects of self-administered stimulants; NMDA receptor antagonists have been reported to decrease or to fail to decrease stimulant self-administration in well-trained animals (review: Beninger and Gerdjikov, 2004).

Self *et al.* (1994) found that inhibition of NAc G proteins Gi and Go with pertussis toxin (PTX) produced long lasting (up to a month) changes in intravenous 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 Gi/Go proteins may be necessary for the rewarding effects of cocaine and heroin. Gi/Go proteins are negatively coupled to the cAMP-PKA pathway, suggesting that an up-regulation of PKA may have resulted in decreased reward and hence higher responding.

Self *et al.* (1998) directly studied the role of NAc 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 of 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 conditioned approach or lever press responding for food (see above) 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 days before drug testing. As it has been shown that this may result in long-term adaptations at the cellular level (e.g., Hyman and Malenka, 2001), it is possible that the functional role of PKA was affected by these changes.

PKA 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 upon 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 (review: Sutton and Beninger, 1999). Some studies have shown that co-injection of amphetamine and an NMDA receptor antagonist leads to a reduction in responding for conditioned reward.

Kelley and Holahan (1997) 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 Gs proteins to receptors in NAc and subsequent increased activation of PKA, that occurs when Gs-coupled receptors are stimulated by DA acting at D_1-like receptors, increases the acquisition of lever press responding for conditioned reward.

In the preceding sections it was noted that Sp-cAMPS injected into the NAc (Baldwin *et al.*, 2002b) or a higher dose into the BLA (Jentsch *et al.*, 2002) impaired acquisition of approach responses to stimuli signaling reward. Similarly, Sp-cAMPS injected into the NAc impaired the acquisition of lever pressing for food (Baldwin *et al.*, 2002b). However, CTX, another agent that augments PKA activity, enhanced the acquisition of approach responses to a stimulus that signaled reward when injected into the BLA (Jentsch *et al.*, 2002) and enhanced the acquisition of responding for conditioned reward when injected into the NAc (Kelley and Holahan, 1997). These results may appear contradictory. However, they may be understood with respect to the specific actions of the agents under study. Thus, Sp-cAMPS would act to increase PKA activity despite the ongoing influence of synaptic input signals at receptors on the cell membrane. On the other hand, CTX, by prolonging the ability of Gs-coupled receptors to stimulate PKA, would preserve the spatio-temporal link between synaptic stimulation and activation of PKA. This may lead to a masking of the reward signal that is critical for reward-related learning by Sp-cAMPS but an augmentation of that signal by CTX. Results showing that Sp-cAMPS impaired the acquisition of reward-related learning whereas CTX enhanced the acquisition of this type of learning are consistent with this hypothesis. A similar argument has been made to account for some of the apparently paradoxical effects of D_1-like DA receptor agonists (Benigner and Miller, 1998; Sutton and Beninger, 1999).

PKA and the Acquisition of a Conditioned Place Preference

The conditioned place preference (CPP) procedure usually involves the pairing of one chamber of a 2- or 3-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 and Glu NMDA receptors in CPP learning. For a thorough review see Tzschentke (1998).

CPP is produced by NAc injections of amphetamine during pairing sessions (Carr and White, 1983; 1986). Beninger *et al.* (2003a) found that co-injections 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 drug-paired side. Co-injections of a sub-threshold dose of amphetamine plus Sp-cAMPS also failed to affect side preference. On the other hand, co-injection 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. Intracerebroventricular (i.c.v.) 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 (Cervo *et al.*, 1997).

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 i.c.v. 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 or lever pressing for food (see above) was impaired by Sp-cAMPS injected into NAc. Results implicate PKA in NAc in reward-related learning in the CPP paradigm.

PKA and the Expression of a Conditioned Place Preference

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 above). For example, CPP based on cocaine was not blocked by 7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5- tetrahydro-(1H)-3-benzazepine hydrochloride (SCH 23390) or sulpiride in the test phase (Cervo and Samanin, 1995). Fewer studies have been done with Glu NMDA receptor antagonists, and both a decrease and no effect has been reported on the expression of CPP (review: Beninger and Gerdjikov, 2004).

One study reported on the effects of H7 injected i.c.v. during testing following conditioning with systemic cocaine. There was no effect on the expression of cocaine CPP (Cervo *et al.*, 1997). Although H7 is a nonspecific serine/threonine kinase inhibitor, this result is consistent with the finding that the expression of conditioned approach responses, lever pressing for food or stimulant self-administration (see above) was not blocked by PKA inhibition.

PKA and the Acquisition or Expression 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 or cocaine. The establishment but not the expression of conditioned activity is blocked by co-administration of DA or Glu NMDA receptor antagonists.

Conditioned activity resulting from pairing NAc amphetamine administration with the test environment was blocked dose-dependently by co-infusion of Rp-cAMPs (Sutton *et al.*, 2000). 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.*, 2003b). Results support previous findings implicating PKA in the acquisition but not the expression of reward-related learning.

Summary of PKA and Reward-related Learning

The results of the PKA studies reviewed above are summarized in Table I. PKA inhibition either following i.c.v. treatments or local injections into the NAc, BLA or mPFC led to a decrease in the acquisition of reward-related learning in at least one of the paradigms considered. In every instance where data were available, a decrease was seen and there were no exceptions. Intra-NAc treatment with the PKA activator Sp-cAMPS also impaired the acquisition of conditioned approach responses, lever pressing for food, and CPP based on NAc amphetamine; intra-BLA Sp-cAMPS similarly impaired acquisition of approach responses at higher doses. On the other hand, intra-BLA CTX enhanced the

acquisition of conditioned approach responses to a cue that signaled reward, and intra-NAc CTX enhanced the acquisition of responding for conditioned reward. As discussed in the section on PKA and the acquisition of lever pressing for conditioned reward above, this difference may be attributable to the different pharmacological actions of Sp-cAMPS and CTX, the former putatively activating PKA indiscriminately and masking the reward signal and the latter putatively augmenting the activation of PKA associated with receptor stimulation, preserving the signal.

With the exception of studies using the self-administration of cocaine to study reward-related learning, in every case where the effects of PKA inhibition (in NAc or following i.c.v. administration) on the expression of reward-related learning were assessed, no effect was seen. Likewise, when PKA activation (in NAc) was tested during the expression of conditioned approach to a stimulus signaling food or lever pressing responses for food no effect was seen. Notwithstanding the exception, results suggest that PKA may play little role in the early expression of reward-related learning once learning has taken place. When considered along with the results from studies of the effects of manipulations of PKA activity during acquisition, results suggest that PKA plays a critical role in the acquisition but not the expression of reward-related learning. It remains to determine the effects of PKA inhibition on repeated testing of established responding; based on the observation that blockade of DA D_1-like receptors leads to a gradual decline in established responding for reward (e.g., Beninger *et al.*, 1987), such tests might be expected to similarly show initial resistance of responding to PKA inhibition followed by a gradual decline with repeated testing.

The one exception to the relatively consistent pattern of results showing that PKA inhibition or indiscriminate activation impairs the acquisition but not the expression of reward-related learning is the effects of Rp-cAMPS and Sp-cAMPS on the expression of lever pressing for cocaine self-administration. Thus, Rp-cAMPS injected into NAc appeared to increase cocaine reward whereas Sp-cAMPS injected into NAc appeared to decrease cocaine reward in well-trained rats (Self *et al.*, 1998). One difference between these self-administration studies and the other studies reviewed above is that the rats had a relatively long history of cocaine intake. It is well known that chronic cocaine treatment leads to compensatory changes in signaling pathways in the brain (Hyman and Malenka, 2001) and it is possible that such changes could alter PKA function, leading to the apparently contradictory results.

Table I Effects of PKA inhibition or activation on acquisition and expression of reward-related learning

		Acquisition		Expression	
Task	Structure	PKA Inhib	PKA Activ	PKA Inhib	PKA Activ
Approach responses	NAc	Decrease	Decrease	No effect	No effect
	BLA	Decrease	Incr/Decr	-	-
	mPFC	Decrease	-	-	-
Lever press for food	NAc	Decrease	Decrease	No effect	No effect
	mPFC	Decrease	-	-	-
Self-admin cocaine	NAc	-	-	Increase	Decrease
Lever press cond rewd	NAc	-	Increase	-	-
CPP to amphetamine	NAc	Decrease	Decrease	-	-
CPP to cocaine	i.c.v.	Decrease	-	No effect	-
Cond act to amphet	NAc	Decrease	-	No effect	-

Abbreviations: Activ = activation; amphet = amphetamine; BLA = basolateral amygdala; cond act = conditioned activity; cond rewd = conditioned reward; CPP = conditioned place preference; Decr = decrease; Incr = increase; Inhib = inhibition; i.c.v. = intracerebroventricular; mPFC = medial prefrontal cortex; NAc = nucleus accumbens; Self-admin = self-administration
Note: dashes (-) indicate that no data are available

PKC AND REWARD-RELATED LEARNING

Ungerer and associates evaluated the effects of treatments on the memory of acquisition of lever pressing for food by injecting drugs after training and assessing their effect on the spontaneous improvement normally seen 24 h later. Using this approach, Stemmelin *et al.* (1999) showed that mice injected i.c.v. with the PKC inhibitor GF 109203X failed to show spontaneous improvement. Results supported a role for PKC in the memory of 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 (Aujla and Beninger, 2003). Similarly, i.c.v. injection of the PKC inhibitor chelerythine immediately after but not before pairing sessions impaired CPP produced by systemic cocaine (Cervo *et al.*, 1997). No CPP was observed following injection of the PKC inhibitors NPC 15437 alone into NAc (Aujla and Beninger, 2003) or calphostin C alone i.c.v. (Narita *et al.*, 2001).

The opioid morphine also has the ability to elicit a robust CPP and this effect requires intact DA transmission (Wise, 1989). Narita *et al.* (2001) found that i.c.v. 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 (Thomas and Everitt, 2001). These authors paired a conditioned stimulus with cocaine injection in a self- administration procedure. Subsequent presentation of the conditioned stimulus alone resulted in up-regulation 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 i.c.v. 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.

MAPK AND REWARD-RELATED LEARNING

The MAPKs include three subfamilies: extracellular signal-regulated kinase (ERK1/2), p38, and c-Jun-N-terminal kinase (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 (Gerdjikov *et al.*, 2004). 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 (Valjent *et al.*, 2000). 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 (Gerdjikov and Beninger, 2004). Unlike Valjent *et al.* (2000), we did not observe a decrease in amphetamine-produced locomotion during conditioning sessions; one difference between these studies was the use of systemic (Valjent *et al.*, 2000) vs. intra-NAc (Gerdjikov and Beninger, 2004) injections of the inhibitor, possibly providing a basis for understanding the observed differences in effects of MAPK inhibition on locomotor activity. Our finding showed that the ability of NAc amphetamine to produce an increase in activity and its ability to produce a CPP can be dissociated.

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.* (2002) found that ERK1 knockout mice showed enhanced striatal ERK2 activation after an i.p. injection of the D_1-like receptor agonist SKF 38393 [(+/-)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol]. Moreover, in contrast to the studies described above, the ERK1 mutants showed an enhanced CPP produced by systemic morphine. Clearly, more research is needed to better understand the separate contributions of ERK1 and ERK2 to reward-related learning.

OTHER SIGNALING MOLECULES AND REWARD-RELATED LEARNING

DARPP-32 and I-1 are PP-1 regulatory proteins localized in the striatum. Zachariou *et al.* (2002) assessed cocaine CPP in DARPP-32 and/or I-1 knockout mice.

DARPP-32, I-1 or mutants lacking both proteins showed impaired CPP to 5 mg/kg i.p. cocaine. At 20 mg/kg, the reduction in CPP was significant only for the double mutant group. Neither group showed reduced unconditioned locomotor activity at these cocaine doses. Results implicate the signaling molecules DARPP-32 and I-1 in the acquisition or expression of a CPP based on cocaine.

Tan (2002) studied the role of CaMKII in amphetamine-produced CPP. Amphetamine conditioning (2.0 mg/kg i.p.) produced a significant CPP and an increase in Ca^{2+}-independent activity of CaMKII in the hippocampus but not the NAc after CPP testing. In addition, intra-hippocampal but not NAc injections of the CaMKII inhibitor KN-93 prior to conditioning sessions with amphetamine impaired the CPP effect. KN-93-injected rats did not show decreased locomotor activity as assessed in an open field test 10 min after drug injection. Results implicated hippocampal but not NAc CaMKII in the acquisition and expression of CPP based on amphetamine.

The role of CREB in reward-related learning has also been tested. Some studies have used the herpes simplex virus as a vector to elevate CREB (HSV-CREB) itself or to elevate a dominant-negative mutant CREB (mCREB) that blocked CREB function in NAc shell. Results revealed that rats treated with HSV-CREB showed elevated CREB in NAc shell and decreased CPP based on cocaine with an aversion being seen at the lowest (threshold) dose of cocaine; conversely, those treated with mCREB showed decreased NAc shell CREB and increased CPP based on cocaine (Carlezon *et al.*, 1998; Pliakas *et al.*, 2001). These results are difficult to reconcile with other findings that suggest that activation of the signaling pathways that lead to increased levels of CREB is associated with increased, not decreased, reward. As was noted above, the one exception to the otherwise consistent pattern of effects of PKA activation or inhibition on the expression of responding for reward was the effects of Rp- and Sp-cAMPS on responding rewarded by cocaine. We suggested that chronic treatment with cocaine might have led to changes in the function of signaling molecules that were reflected in the apparently contradictory findings. In the studies with HSV-CREB and mCREB, the researchers were seeking to assess changes in CREB that were like those that might be expected to be seen in animals chronically treated with cocaine. Thus, although the rats in that study were not rats with a history of chronic cocaine self-administration, they were rats that received treatments intended to mimic some of the molecular consequences of chronic cocaine use. Results appeared to complement those of the study of Self *et al.* (1998) and, as in that study, may reflect compensatory changes in signal molecular function brought about by chronic drug abuse rather than the signaling pathways normally involved in reward-related learning. A reconciliation of the results from studies that manipulate CREB activity in the NAc shell with those of other experiments showing that decreased activity in the cAMP-PKA signaling cascade (that would lead to decreased CREB activation) leads to decreased reward (see above) will have to await further study.

A MODEL OF THE MOLECULAR MECHANISMS OF REWARD-RELATED LEARNING

In recent years, the molecular mechanisms of learning have been extensively studied. This work is exemplified by investigations of the sea slug *Aplysia* (Kandel, 2001). However, numerous other species have been studied. Thus, somewhat similar mechanisms have been identified in *Caenorhabditis Elegans* (Rankin, 2002), *Drosophila* (Waddell and Quinn, 2001), honey bee (Fiala *et al.*, 1999), chick (Rose, 2000) and rat (Izquierdo and Medina, 1997; Izquierdo *et al.*, 1999; Kandel, 2001). 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.

Role of Glutamate and Dopamine in the Striatum and Nucleus Accumbens

Beninger (1983) first proposed a synaptically based mechanism for reward-related learning. He proposed a heterosynaptic facilitation model involving the ability of DA afferents, acting at D_1-like receptors, to modulate co-terminating cholinergic afferents on medium spiny striatal 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 (1988; 1990; 1993) and his co-workers (Miller *et al.*, 1990; Wickens and Kötter, 1995) 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 (Cepada and Levine, 1998; Nicola *et al.*, 2000; Reynolds and Wickens, 2000; Centonze *et al.*, 2001; Reynolds *et al.*, 2001).

The model can be summarized as follows (cf.,

Beninger, 1993): Most of the neurons in the striatum (including the NAc) are of the medium spiny type. These neurons use γ-aminobutyric acid (GABA) as their principle neurotransmitter and are the principle projection neurons of the striatum (Kita and Kitai, 1988). The spines of these neurons receive Glu inputs from cortical neurons and DA inputs from mesencephalic neurons (Smith and Bolam, 1990). Among other things, cortical inputs carry information about the perception of stimuli in the environment, and the output neurons of the striatum influence motor action. DA, by modifying the strength of Glu synapses in the striatum, 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; i.e., at Glu synapses that are in a state of readiness (Miller, 1981). As outlined in the next section, the molecular events underlying DA-mediated learning are beginning to yield to the efforts of researchers.

Role of Signaling Molecules in Reward-related Learning

Several recent reviews provide an extensive look at the molecular signaling cascades that are thought to mediate the modulating influence of DA on Glu synapses (Kelley, 1999; Sutton and Beninger, 1999; Kelley and Berridge, 2002). 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 (1990) proposed that this event might represent the state of readiness posited by Miller (1981). Increased spine $[Ca^{2+}]$ leads to activation of enzymes including PKC and CaMKII (Lisman *et al.*, 2002); these enzymes phosphorylate a variety of proteins including, for example, Glu α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors, altering their synaptic anchoring and open time, and are known to be necessary for some of the molecular signals produced by stimulation of D_1-like DA receptors (Konradi *et al.*, 1996; Das *et al.*, 1997; Lisman *et al.*, 2001). One effect of increased $[Ca^{2+}]$ is enhanced coupling of adenylyl cyclase (AC) to G-protein-coupled receptors such as the D_1-like DA receptor (Gnegy, 1982; Abrams *et al.*, 1991; Yovell *et al.*, 1992; Xia *et al.*, 1995). This event would enhance the impact of subsequent DA inputs on signaling. In the absence of a DA input in close temporal contiguity with the Glu input that establishes this state of readiness, the enzyme protein PP-1 will dephosphorylate recently phosphorylated proteins and $[Ca^{2+}]$ will fall, undoing the putative state of readiness.

When reward occurs and DA is released, stimulation of D_1-like receptors will lead to enhanced activation of AC because of the enhanced coupling of AC with D_1-like receprtors, formation of cAMP and stimulation of PKA. PKA phosphorylates DARPP-32 that, in turn, inhibits PP-1. 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 CREB, a transcription factor involved in gene expression. CREB activation requires not only PKA but also stimulation of NMDA receptors and increases in $[Ca^{2+}]$ (Konradi *et al.*, 1996; Das *et al.*, 1997); this makes it an excellent candidate for mediating temporally contiguous activation of DA and Glu receptors on synaptic spines of medium spiny neurons (FIG. 1).

These are only a few of the many molecular events that occur upon stimulation of DA or Glu receptors. For example, the MAPK ERK1/2 has been implicated in learning and memory (Adams and Sweatt, 2002); 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 (Vincent *et al.*, 1998). JNK is another MAPK that phosphorylates activating transcription factor 2 (ATF-2), a CREB family member (Curtis and Finkbeiner, 1999). The ability of amphetamine to activate MAPKs has been found to depend on mGluRs (Choe *et al.*, 2002). These kinases also might play a role in reward-related learning.

A CONSIDERATION OF THE EXPERIMENTAL RESULTS FROM THE POINT OF VIEW OF THE MODEL

The model proposes that the activation of PKA in striatal neurons that receive cortical synapses that results from the stimulation by DA of D_1-like receptors in association with the presentation of a rewarding stimulus is a critical event in the acquisition of reward-related learning. There is ample evidence that injections into the NAc of PKA inhibitors block the acquisition of reward-related learning in a number of paradigms, supporting this aspect of the model (Table I). The observa-

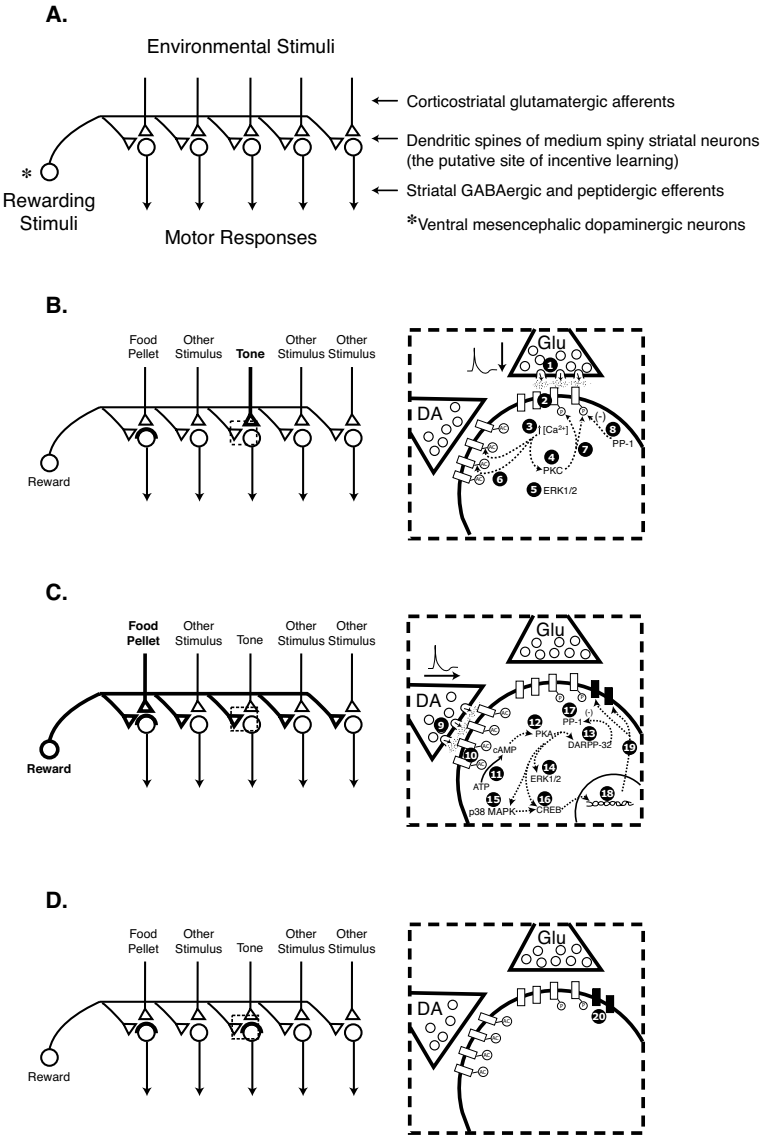

FIGURE 1 Possible role of signaling molecules in reward-related incentive learning. **A**: Dorsal and ventral (nucleus accumbens) striatal medium spiny GABAergic and peptidergic efferent neurons influence motor responses. Individual spines on medium spiny neuronal dendrites receive glutamatergic (Glu) inputs from cortical neurons, activated by environmental stimuli, and dopaminergic (DA) inputs from ventral mesencephalic neurons, activated by rewarding stimuli. Incentive learning may occur as a result of DA-mediated changes in the strength of Glu synapses made by cortical efferents onto medium spiny neuron dendritic spines. **B**: When environmental stimuli (e.g., tone) are encountered, activation of corticosriatal projections will lead to the release of Glu (1 in inset) and stimulation of Glu receptors (2). Glu (*N*-methyl-D-aspartate) receptor stimulation leads to increased local intracellular calcium concentrations [Ca^{2+}] (3), activation of Ca^{2+}-dependent protein kinase (PKC) (4) and activation of the ERK1/2 (5). Increased [Ca^{2+}] may enhance the coupling of D_1-like DA receptors to adenylyl cyclase (AC) (6) and PKC may lead to phosphorylation (P) of α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA)-type Glu receptors (7) leading to increased open time when they are stimulated. In the absence of a DA input, protein phosphatase-1(PP-1) will dephosphorylate recently phosphorylated proteins (8). **C**: If a rewarding stimulus such as food is encountered immediately after the tone stimulus, the food stimulus will activate sensory pathways leading to activation of corticostriatal Glu projections, and reward pathways leading to activation of mesostriatal DA neurons and synaptic release of DA (9 in inset). Stimulation of D_1-like receptors by DA will lead to enhanced activation of AC (10), leading to conversion of adenosine triphosphate (ATP) to cyclic 3',5'-adenosine monophosphate (cAMP) (11). cAMP, in turn, activates PKA (12) which activates DA and cAMP-regulated phosphoprotein-32 kDa (DARPP-32) (13), ERK1/2 (14), p38 MAPK (15) and cAMP response element-binding proteins (CREB) (16). CREB is also activated by p38 MAPK. DARPP-32 inhibits protein phosphatase 1 (PP-1) (17), thereby retarding the dephosphorylation of proteins, e.g., AMPA receptors recently phosphorylated as a result of increases in local [Ca^{2+}]; this may constitute a form of short term incentive learning. PKA activated CREB influences gene transcription and new protein synthesis (18). Newly synthesized proteins may influence Glu receptors and thereby Glu synaptic effectiveness (19). **D**: After incentive learning has taken place, strengthened Glu synapses (solid rectangles at 20 in inset) can influence motor responses, at least transiently, in the absence of DA or PKA.

tion that injections into the NAc of a PKA activator similarly impaired learning is also consistent with the model if these treatments, by indiscriminately activating PKA, occlude the signal normally produced by reward (as proposed above). Some experiments showed that PKA inhibition in the BLA or mPFC impaired learning. These results implicate signaling molecules in these structures in reward-related learning but whether the type of synaptic interaction that has been proposed for the NAc also occurs in these other structures is presently unclear. In summary, there is good evidence that activation of PKA in striatal neurons is necessary for the acquisition of reward-related learning.

With the exception of cocaine self-administration studies (see above), results of tests of the effects of intra-NAc injections of PKA inhibitors or activators on the expression of reward-related learning were negative. These findings are consistent with the observations from many studies showing that DA is necessary for the acquisition of reward-related learning but once learning has taken place it is transiently resistant to the effects of DA receptor antagonists.

According to the model, one of the substrates for PKA is DARPP-32, its phosphorylation leading to its ability to inhibit PP-1 and thereby preserve the newly phophorylated proteins (e.g., AMPA receptors) within the synaptic spine. These proteins would have been phosphorylated as a result of stimulation of NMDA receptors and consequent increases in $[Ca^{2+}]$. Results showing that DARPP-32 knock out mice were impaired in learning a CPP based on cocaine are consistent with this model. Perhaps this aspect of the model represents a component of reward-related learning that is relatively short term and that is not dependent on protein synthesis. Another substrate for PKA is CREB. To our knowledge, the only study that looked at changes in CREB in conjunction with tests of reward-related learning used a relatively long-term treatment designed to mimic some of the effects of chronic drug intake on CREB in NAc. The results of this study were not consistent with the model, suggesting that activation of CREB is part of the signaling pathway mediating the learning effects of reward and D_1-like receptor stimulation although they do implicate CREB in learning. It will be the task of future studies to further evaluate the role of CREB in reward-related learning.

A number of studies have shown that PKC in NAc is involved in the acquisition of reward-related learning. PKC inhibitors have been found to impair NAc amphetamine-produced CPP, for example, and increased levels of a PKC isoform have been found in NAc following appetitive conditioning. One study looked at the effects of CaMKII inhibition in NAc on the acquisition of an amphetamine CPP and found no effect but their results showed that CaMKII inhibition in the hippocampus impaired learning in this task. Results with PKC inhibition are consistent with the model insofar as it posits that striatal Glu inputs produced by stimuli that signal reward lead to increased $[Ca^{2+}]$ in striatal neurons that in turn leads to activation of Ca^{2+}-sensitive enzymes including PKC. The particular substrates of PKC that are relevant to its role in reward-related learning, to our knowledge, remain to be elucidated. Further studies are needed to assess the role of NAc CaMKII in reward-related learning.

The MAPKs ERK1/2 and p38 but not JNK in NAc have been implicated in the acquisition of reward-related learning using CPP based on NAc amphetamine. The model that has been proposed for how DA and Glu synaptic inputs on striatal neurons might interact does not include MAPKs. On the other hand, MAPKs influence CREB activity and CREB is thought to be in the pathway from PKA to the transcription of genes (see Kelley and Berridge, 2002). It remains the task of future researchers to work out the complex interplay of signaling molecules that leads to reward-related learning.

CONCLUSIONS

Knowledge about signaling molecules and their multiple intracellular actions and interactions continues to expand rapidly. There is now good evidence that both DA and Glu are involved in reward-related learning and a model has been developed for how a conjunction of synaptic inputs from cells using these neurotransmitters might lead to eventual long-term changes in Glu synaptic efficacy. This model utilizes information about the effects of these inputs on signaling molecular cascades and their interactions to provide a basis for understanding the mechanisms underlying reward-related learning. Already, there is strong evidence for a role for PKA and mounting evidence that DARPP-32, CREB, PKC, CaMKII and MAPKs are involved. Further studies are sure to continue to elucidate the role of signaling molecules in reward-related learning. The neurotransmitters implicated in reward-related learning, DA and Glu, also have been implicated in neuropsychiatric disorders including Parkinson's disease and schizophrenia and in drug abuse. As we further develop an understanding of the molecular signaling pathways that mediate the role of these neurotransmitters in learning and memory, we will be better equipped to bring new and innovative approaches to the treatment of these diseases.

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References

Abrams TW, KA Karl and ER Kandel (1991) Biochemical studies of stimulus convergence during classical conditioning in Aplysia, dual regulation of adenylate cyclase by Ca^{2+}/calmodulin and transmitter. *J. Neurosci.* **11**, 2655-2665.

Adams JP and JD Sweatt (2002) Molecular psychology, roles for the ERK MAP kinase cascade in memory. *Annu. Rev. Pharmacol. Toxicol.* **42**, 135-163.

Aujla H and RJ Beninger (2003) Intra-accumbens protein kinase C inhibitor NPC 15437 blocks amphetamine-produced conditioned place preference in rats . *Behav. Brain Res.* **147**, 41-48.

Baldwin AE, K Sadeghian and AE Kelley (2002a) Appetitive instrumental learning requires coincident activation of NMDA and dopamine D_1 receptors within the medial prefrontal cortex . *J. Neurosci.* **22**, 1063-1071.

Baldwin AE, K Sadeghian, MR Holahan and AE Kelley (2002b) Appetitive instrumental learing is impaired by inhibition of cAMP-dependent protein kinase within the nucleus accumbens. *Neurobiol. Learn. Mem.* **77**, 44-62.

Beninger RJ (1983) The role of dopamine in locomotor activity and learning. *Brain Res. Rev.* **6**, 173-196.

Beninger RJ (1993) Role of D_1 and D_2 receptors in learning, In *D_1,D_2 Dopamine Receptor Interactions, Neuroscience and Pharmacology* (Waddington J, Ed.) (Academic Press: London), pp 115-157.

Beninger RJ and TV Gerdjikov (2004) Dopamine-glutamate interactions in reward-related incentive learning, In *Dopamine and Glutamate in Psychiatric Disorders* (Schmidt WJ and MEA Reith, Eds.) (Humana Press Inc.: Totowa, NJ) in press.

Beninger RJ and R Miller (1998) Dopamine D_1-like receptors and reward-related incentive learning. *Neurosci. Biobehav. Rev.* **22**, 335-345.

Beninger RJ, M Cheng, BL Hahn, DC Hoffman, EJ Mazurski, MA Morency, P Ramm and RJ Stewart (1987) Effects of extinction, pimozide, SCH 23390, and metoclopramide on food-reinforced operant responding. *Psychopharmacology (Berl.)* **92**, 343-349.

Beninger RJ, PL Nakonechny and I Savina (2003a) cAMP-dependent protein kinase and reward-related learning, intra-accumbens Rp-cAMPS blocks amphetamine-produced place conditioning in rats. *Psychopharmacology (Berl.)* **170**, 23-32.

Beninger RJ, J Wiesenthal and AC Giles (2003b) Conditioned locomotion based on nucleus accumbens amphetamine, enhancement by PKA inhibitor Rp-cAMPS. *Behav. Pharmacol.* **14**, Suppl. 1, S73.

Berridge KC and TE Robinson (1998) What is the role of dopamine in reward, hedonic impact, reward learning, or incentive salience? *Brain Res. Rev.* **28**, 309-369.

Bindra D (1974) A motivational view of learning, performance and behavior modification. *Psychol.Rev.* **81**, 199-213.

Carlezon WA Jr, J Thome, VG Olson, SB Lane-Ladd, ES Brodkin, N Hiroi, RS Duman, RL Neve and EJ Nestler (1998) Regulation of cocaine reward by CREB. *Science* **282**, 2272-2275.

Carr GD and NM White (1983) Conditioned place preference from intra-accumbens but not intra-caudate amphetamine injections. *Life Sci.* **33**, 2551-2557.

Carr GD and NM White (1986) Anatomical disassociation of amphetamine's rewarding and aversive effects, an intracranial microinjection study. *Psychopharmacology (Berl.)* **89**, 340-346.

Centonze D, B Picconi, P Gubellini, G Bernardi and P Calabresi (2001) Dopaminergic control of synaptic plasticity in the dorsal striatum. *Eur. J. Neurosci.* **13**, 1071-1077.

Cepeda C and MS Levine (1998) Dopamine and *N*-methyl-D-aspartate receptor interactions in the neostriatum. *Dev. Neurosci.* **20**, 1-18.

Cervo L and R Samanin (1995) Effects of dopaminergic and glutamatergic receptor antagonists on the acquisition of cocaine conditioned place preference. *Brain Res.* **673**, 242-250.

Cervo L, S Mukherjee, A Bertaglia and R Samanin (1997) Protein kinases A and C are involved in the mechanisms underlying consolidation of cocaine place conditioning. *Brain Res.* **775**, 30-36.

Choe ES, KT Chung, L Mao and JQ Wang (2002) Amphetamine increases phosphorylation of extracellular signal-regulated kinase and transcription factors in the rat striatum via group I metabotropic glutamate receptors. *Neuropsychopharmacology* **27**, 565-575.

Curtis J and S Finkbeiner (1999) Sending signals from the synapse to the nucleus, possible roles for CaMK, Ras/ERK, and SAPK pathways in the regulation of synaptic plasticity and neuronal growth. *J. Neurosci. Res.* **58**, 88-95.

Das S, M Grunert, L Williams and SR Vincent (1997) NMDA and D_1 receptors regulate the phosphorylation of CREB and the induction of c-fos in striatal neurons in primary culture. *Synapse* **25**, 227-233.

Fiala A, U Muller and R Menzel (1999) Reversible downregulation of protein kinase A during olfactory learning using antisense technique impairs long-term memory formation in the honeybee, *Apis mellifera*. *J. Neurosci.* **19**, 10125-10134.

Gerdjikov TV, GM Ross and RJ Beninger (2004) Place preference induced by nucleus accumbens amphetamine is impaired by antagonists of ERK or p38 MAP kinases in rats. *Behav. Neurosci.* in press

Gnegy ME (1982) Relationship of calmodulin and dopaminergic activity in the striatum. *Fed. Proc.* **41**, 2273-2277.

Hitchcott PK and GD Phillips (1998) Double dissociation of the behavioural effects of *R*(+) 7-OH-DPAT infusions in the central and basolateral amygdala nuclei upon Pavlovian and instrumental conditioned appetitive behaviours. *Psychopharmacology (Berl.)* **140**, 458-469.

Hyman SE and RC Malenka (2001) Addiction and the brain, the neurobiology of compulsion and its persistence. *Nat. Rev. Neurosci.* **2**, 695-703.

Izquierdo I and JH Medina (1997) Memory formation, the sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. *Neurobiol. Learn. Mem.* **68**, 285-316.

Izquierdo I, JH Medina, MRM Vianna, LA Izquierdo and DM Barros (1999) Separate mechanisms for short- and long-term memory. *Behav. Brain Res.* **103**, 1-11.

Jentsch JD, P Olausson, EJ Nestler and JR Taylor (2002) Stimulation of protein kinase A activity in the rat amygdala enhances reward-related learning. *Biol. Psychiatry* **52**, 111-118.

Kandel E (2001) The molecular biology of memory storage, a dialogue between genes and synapses. *Science* **294**, 1030-1038.

Kelley AE (1999) Neural integrative activities of nucleus accumbens subregions in relation to learning and motivation. *Psychobiol*. **27**, 198-213.

Kelley AE and KC Berridge (2002) The neuroscience of natural rewards, relevance to addictive drugs.[comment]. *J. Neurosci.* **22**, 3306-3311.

Kelley AE and MR Holahan (1997) Enhanced reward-related responding following cholera toxin infusion into the nucleus accumbens. *Synapse* **26**, 46-54.

Kita H and ST Kitai (1988) Glutamate decarboxylase immunoreactive neurons in rat neostriatum, their morphological types and populations. *Brain Res.* **447**, 346-352.

Konradi C, JC Leveque and SE Hyman (1996) Amphetamine and dopamine-induced immediate early gene expression in striatal neurons depends on postsynaptic NMDA receptors and calcium. *J. Neurosci.* **16**, 4231-4239.

Lisman JE and AM Zhabotinsky (2001) A model of synaptic memory, a CaMKII/PP1 switch that potentiates transmission by organizing an AMPA receptor anchoring assembly. *Neuron* **31**, 191-201.

Lisman J, H Schulman and H Cline (2002) The molecular basis of CaMKII function in synaptic and behavioural memory. *Nat. Rev. Neurosci.* **3**, 175-190.

Mazzucchelli C, C Vantaggiato, A Ciamei, S Fasano, P Pakhotin, W Krezel, H Welzl, DP Wolfer, G Pages, O Valverde, A Marowsky, A Porrazzo, PC Orban, R Maldonado, MU Ehrengruber, V Cestari, HP Lipp, PF Chapman, J Pouyssegur and R Brambilla (2002) Knockout of ERK1 MAP kinase enhances synaptic plasticity in the striatum and facilitates striatal-mediated learning and memory. *Neuron* **34**, 807-820.

Miller R (1981) *Meaning and Purpose in the Intact Brain* (Clarendon Press: Oxford, UK).

Miller R, JR Wickens and RJ Beninger (1990) Dopamine D-1 and D-2 receptors in relation to reward and performance, A case for the D-1 receptor as a primary site of therapeutic action of neuroleptic drugs. *Prog. Neurobiol.* **34**, 143-183.

Narita M, T Aoki, S Ozaki, Y Yajima and T Suzuki (2001) Involvement of protein kinase Cγ isoform in morphine-induced reinforcing effects. *Neuroscience* **103**, 309-314.

Nicola SM, DJ Surmeier and RC Malenka (2000) Dopaminergic modulation of neuronal excitability in the striatum and nucleus accumbens. *Annu. Rev. Neurosci.* **23**, 185-215.

Pliakas AM, RR Carlson, RL Neve, C Konradi, EJ Nestler and WA Carlezon Jr (2001) Altered responsiveness to cocaine and increased immobility in the forced swim test associated with elevated cAMP response element-binding protein expression in nucleus accumbens. *J. Neurosci.* **21**, 7397-7403.

Rankin CH (2002) From gene to identified neuron to behaviour in *Caenorhabditis elegans*. *Nat. Rev. Genetics* **3**, 622-630.

Reynolds JNJ and JR Wickens (2000) Substantia nigra dopamine regulates synaptic plasticity and membrane potential fluctuations in the rat neostratum, *in vivo*. *Neuroscience* **99**, 199-204.

Reynolds JN, BI Hyland and JR Wickens (2001) A cellular mechanism of reward-related learning. *Nature* **413**, 67-70.

Rose SPR (2000) God's organism? The chick as a model system for memory studies. *Learn. Mem.* **7**, 1-17.

Self DW, RZ Terwilliger, EJ Nestler and L Stein (1994) Inactivation of Gi and Go proteins in nucleus accumbens reduces both cocaine and heroin reinforcement. *J. Neurosci.* **14**, 6239-6247.

Self DW, LM Genova, BT Hope, WJ Barnhart, JJ Spencer and EJ Nestler (1998) Involvement of cAMP-dependent protein kinase in the nucleus accumbens in cocaine self-administration and relapse of cocaine-seeking behavior. *J. Neurosci.* **18**, 1848-1859.

Smith AD and JP Bolam (1990) The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurons. *Trends Neurosci.* **13**, 259-265.

Stemmelin J, C Mathis and A Ungerer (1999) GF 109203X, a selective inhibitor of protein kinase C, impairs retention performance in an operant task. *Neuroreport* **10**, 2805-2809.

Sutton MA and RJ Beninger (1999) Psychopharmacology of conditioned reward, evidence for a rewarding signal at D_1-like dopamine receptors. *Psychopharmacology (Berl.)* **144**, 95-110.

Sutton MA, K McGibney and RJ Beninger (2000) Conditioned locomotion in rats following amphetamine infusion into the nucleus accumbens, blockade by coincident inhibition of protein kinase A. *Behav. Pharmacol.* **11**, 365-376.

Tan SE (2002) Impairing the amphetamine conditioning in rats through the inhibition of hippocampal calcium/calmodulin-dependent protein kinase II activity. *Neuropharmacology* **42**, 540-547.

Thomas KL and BJ Everitt (2001) Limbic-cortical-ventral striatal activation during retrieval of a discrete cocaine-associated stimulus, a cellular imaging study with γ protein kinase C expression. *J. Neurosci.* **21**, 2526-2535.

Tzschentke TM (1998) Measuring reward with the conditioned place preference paradigm, a comprehensive review of drug effects, recent progress and new issues. *Prog. Neurobiol.* **56**, 613-672.

Valjent E, JC Corvol, C Pages, MJ Besson, R Maldonado and J Caboche (2000) Involvement of the extracellular signal-regulated kinase cascade for cocaine-rewarding properties. *J. Neurosci.* **20**, 8701-8709.

Vincent SL, M Sebben, A Dumuis and J Bockaert (1998) Neurotransmitter regulation of MAP kinase signaling in striatal neurons in primary culture. *Synapse* **29**, 29-36.

Waddell S and WG Quinn (2001) Flies, genes, and learning. *Annu. Rev. Neurosci.* **24**, 1283-1309.

Wickens J (1988) Electrically coupled but chemically isolated synapses, dendritic spines and calcium in a rule for synaptic modification. *Prog. Neurobiol.* **31**, 507-528.

Wickens J (1990) Striatal dopamine in motor activation and reward-mediated learning, steps towards a unifying model. *J. Neural Transm.* **80**, 9-31.

Wickens JR (1993) *A Theory of the Striatum* (Pergamon: Tarrytown, NY).

Wickens J and R Kötter (1995) Cellular models of reinforcement, In *Models of Information Processing in the Basal Ganglia* (Houk JC, J Davis and DG Beiser, Eds.) (MIT Press: Cambridge, MA) pp 187-214.

Wise RA (1989) Opiate reward: sites and substrates. *Neurosci. Biobehav. Rev.* **13**, 129-133.

Xia ZG, EJ Choi, DR Storm and C Blazynski (1995) Do the calmodulin-stimulated adenylyl cyclases play a role in neuroplasticity? *Behav. Brain Sci.* **18**, 429-440.

Yovell Y, ER Kandel, Y Dudai and TW Abrams (1992) A quantitative study of the [Ca^{2+}]-calmodulin sensitivity of adenylyl cyclase in Aplysia, Drosophila, ansd rat. *J. Neurochem.* **59**, 1736-1744.

Zachariou V, M Benoit-Marand, PB Allen, P Ingrassia, AA Fienberg, F Gonon, P Greengard and MR Picciotto (2002) Reduction of cocaine place preference in mice lacking the protein phosphatase 1 inhibitors DARPP 32 or Inhibitor 1. *Biol. Psychiatry* **51**, 612-620.