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Dopaminergic Mechanism of Reward-Related Incentive Learning: Focus on the Dopamine D_3 Receptor

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Dopamine D_3 receptors (Drd3) have been implicated in the control of responding by drug-related conditioned incentive stimuli. We review recent studies of the effects of Drd3 antagonists or partial agonists on the control of self-administration of intravenous (IV) cocaine, IV morphine and oral ethanol on reward-rich and lean schedules, in reinstatement tests, on second-order schedules and on the acquisition and expression of conditioned place preference (CPP) and conditioned motor activity. For comparison, related studies where conditioned stimuli are based on nutritional reward also are considered. When self-administration depends more heavily on conditioned cues for its maintenance, for example on second-order schedules or lean ratio schedules, Drd3 antagonists or partial agonists reduce responding. Although data are limited, similar effects may be seen for responding for cues based on drugs or nutritional rewards. Drd3 agents also block the ability of conditioned cues to reinstate responding for cocaine or food. Published results suggest that Drd3 plays a more important role in the expression than in the acquisition of a CPP or conditioned motor activity. The mechanism mediating the role of Drd3 in the control of responding by conditioned incentive stimuli remains unknown but it has been found that Drd3 receptors increase in number in the nucleus accumbens during conditioning. Perhaps Drd3 participates in the molecular mechanisms underlying the role of dopamine and of dopamine receptor subtypes in reward-related incentive learning.

Keywords: ABT-127; BP 897; CJB 090; NGB 2904; PNU 99194-A; RGH-237; SB-277011-A; ST 198

INTRODUCTION

Rewarding stimuli produce incentive learning, increasing the ability of neutral stimuli to elicit approach and other responses (Bolles, 1972; Bindra, 1978; Beninger, 1983). Conditioned incentive stimuli can also act as rewarding stimuli in their own right. Dopamine (DA) has been implicated in this form of learning (Beninger, 1983; Robbins and Everitt, 1996; Berridge and Robinson, 1998; DiChiara, 1999). Various DA receptor subtypes have been shown to play a role in some aspects of incentive learning but the mechanisms remain to be fully worked out (*e.g.*, Beninger and Gerdjikov, 2004). In recent years the development of new,

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Table I Dopamine D_3 receptor (Drd3) antagonists and partial agonists showing relative binding affinity ratio of Drd2 *vs* Drd3. Increasing numbers indicate increasing preference for Drd3

Compound	Receptor Action	$K_{i(Drd2)}/K_{i(Drd3)}$	References
ABT-127	Antagonist	148	(Unger *et al.*, 2005)
NGB 2904	Antagonist	56-830	(Xi and Gardner, 2007)
PNU 99194-A	Antagonist	10-14	(Heidbreder *et al.*, 2005)
SB-277011-A	Antagonist	79-263	(Heidbreder *et al.*, 2005)
ST 198	Antagonist	62-65	(Bezard *et al.*, 2003; Mach *et al.*, 2004)
BP 897	Partial Agonist	66	(Heidbreder *et al.*, 2005)
CJB 090	Partial Agonist	50	(Martelle *et al.*, 2007)
RGH-237	Partial Agonist	1800	(Gyertyan *et al.*, 2007)

more selective pharmacological agents has made it possible to begin to evaluate the role of DA D_3 receptors (Drd3) (Table I). The purpose of the present paper is to review recent studies of the role of Drd3 in incentive learning.

DA exerts its actions via two families of receptors defined on the basis of their pharmacological profile, function and homology: the DA D_1-like receptor (Drd1-like) family includes Drd1 and Drd5 and the Drd2-like family includes the Drd2, Drd3 and Drd4 (Vallone *et al.*, 2000). The stimulation of Drd1-like leads to activation of adenylyl cyclase and the cyclic adenosine monophosphate (cAMP)-cAMP-dependent protein kinase (PKA) cascade (Stoof and Kebabian, 1981). They are found primarily in the cortex and hippocampus and also in the caudate and nucleus accumbens (NAc). Drd2, Drd3 and Drd4 are coupled to inhibitory G proteins that inhibit adenylyl cyclase and in turn the cAMP-PKA cascade. Drd2, Drd3 and Drd4 are found in a number of brain regions including the caudate, putamen and NAc. Drd3 is found primarily in limbic regions with moderate to high concentrations in the Isles of Calleja, NAc, olfactory tubercle and basolateral amygdala (BLA) (Levant, 1997; Vallone *et al.*, 2000).

A number of behavioural test procedures have been used to study the role of Drd3 in incentive learning. In some cases, the effects of Drd3-specific agents on the *expression* of learning that has already taken place have been studied and in other cases these same agents have been evaluated during *acquisition*. This has proven to be an important distinction for the study of Drd3 function.

In the following we will review the effects of Drd3 antagonists and partial agonists on: i) responding maintained by the intravenous (IV) infusion of rewarding drugs including cocaine and nicotine, ii) place conditioning and iii) conditioned motor activity. Heidbreder *et al.* (2005) have previously reviewed some of these results. In the final section we will discuss the implications of these results for the putative mechanisms underlying the role of DA receptors in incentive conditioning.

Drd3 Antagonists and Drug Self-Administration

Self-administration experiments sometimes begin with training rats to press a lever for a conventional reward such as food or water (Roberts and Goeders, 1989). When training animals to respond for food, magazine training often takes place prior to lever press training (*e.g.*, Mackintosh, 1974, p. 146). This involves presenting food pellets non-contingently into the food cup so that the rat learns the association between the sound of operating the food magazine and the presentation of a food pellet. The animal also learns the location of the food. Because they precede the delivery of food, the magazine sound and the food cup will acquire incentive value, thereby having an increased ability to elicit approach and other responses (*e.g.*, lever pressing; Bindra, 1978). The rat will gradually learn that the food cup does not predict food on its own but only when the magazine sound occurs. The magazine sound will also acquire the ability to act as a conditioned reward, producing activation of DA neurons

in its own right (Schultz *et al.*, 1997) and be able to strengthen the incentive value of stimuli that precede it. Thus, after magazine training and during lever-press response shaping, the temporal contiguity between being near the lever and hearing the sound will lead to the lever acquiring incentive value. During response shaping, the rat will repeatedly return to the location of the lever as the association between being there and receiving food is strengthened (Reynolds, 1968, p. 27). As the incentive value of the lever increases the rat will continue to approach and manipulate it until it eventually learns that the application of downward pressure on the lever leads to food. Thus, a lever press response is shaped (see Reynolds, 1968, pp. 26-31).

Animals outfitted with IV cannulae that have been trained to lever press for food can have the cannulae connected to an infusion pump so that lever presses lead to a drug infusion instead of food. These animals learn quickly to lever press for cocaine, nicotine or morphine (Roberts and Goeders, 1989). The drug normally is signaled by a light stimulus that lasts for several seconds, often the duration of the infusion; the animals may also hear the sound of the infusion pump operating. These cues become conditioned rewards because of their association with the drug (Davis and Smith, 1979; deWit and Stewart, 1981). These conditioned rewards can then stimulate DA release (Fontana *et al.*, 1993; Kiyatkin and Stein, 1996; Ito *et al.*, 2000) and can maintain responding for long periods without the drug as long as they are periodically associated with the drug as seen, for example, in second-order schedules (Goldberg, 1973; Whitelaw *et al.*, 1996; Arroyo *et al.*, 1998). It is also possible for rats to learn from the outset to lever press for IV drug even without response shaping by the experimenter (Roberts and Goeders, 1989). If they spontaneously press the lever, a cue is presented and a drug infusion follows. The subsequent enhanced levels of synaptic DA increase the incentive value of the cue that then operates as a conditioned reward for lever pressing (Bindra, 1978).

In a number of studies, Drd3 antagonists have no significant effect on lever pressing for drug when the schedule of reward presentation is a reward-rich fixed ratio (FR) 1 or 2; this was shown for cocaine in rats with the antagonists SB-277011-A (trans-*N*-[4-[2-(6-cyano-1,2,3,4-tetrahydroisoquinolin-2-yl) ethyl]syclohexyl]4-quinolininecarboxamide]) (Di Ciano *et al.*, 2003; Gal and Gyertyan, 2003; Xi *et al.*, 2005) and NGB 2904 (*N*-(4-[4-{2,3-dichlorophenyl}-1-piperazinyl]butyl)-2-fluorenyl-carboxamide) (Xi *et al.*, 2006; Xi and Gardner, 2007) and for nicotine with SB-277011-A (Ross *et al.*, 2007). On the other hand, the choice to self-administer or lever press on a FR1 for oral ethanol by rats or mice decreased after SB-277011-A (Thanos *et al.*, 2005; Heidbreder *et al.*, 2007). Thus, Drd3 antagonists have no significant effect on lever pressing for cocaine or nicotine when the schedule of reward presentation is a high density FR but appear to affect choice to consume or lever press on a FR1 for ethanol.

The partial Drd3 agonists BP-897 (1-(4-(2-naphthoyl-amino)butyl)-4-(2-methoxyhenyl)-1A-piperazine hydrochloride) and RGH-237 [*N*-{4-[4-(3-aminocarbonyl-phenyl)-piperazin-1-yl]-butyl}-4-bromo-benzamide], like the full Drd3 antagonists SB-277011-A and NGB 2904, failed to significantly affect FR1 responding for IV cocaine (Pilla *et al.*, 1999; Gyertyan *et al.*, 2007). Partial DA agonists are thought to moderately stimulate the receptor during times when endogenous DA tone is lacking or diminished but to antagonize the receptor from full activation during conditions of increased DA release associated with drug seeking or taking (Gyertyan *et al.*, 2007). Thus, BP-897 and RGH-237 might act like Drd3 antagonists in drug self-administration experiments.

When the rewarding drug stimulus follows every response or every second response, the behaviour depends relatively less on conditioned incentive stimuli. Responding can be made to depend more heavily on incentive cues by instituting a second-order schedule of reward (Kelleher, 1966). On these potentially reward-lean schedules responding is intermittently rewarded by a conditioned rewarding stimulus and the primary reward is only presented after a number of components of responding for the conditioned stimulus are completed. For example, in a FI 15-min (FR10:S) second-order schedule, every tenth response is rewarded by a brief conditioned stimulus (S) and the first FR10 to be completed after a fixed interval (FI) of 15 min has elapsed is followed by a conditioned stimulus plus

the primary reward, an IV infusion of drug. Responding on a second-order schedule depends more heavily on incentive cues; indeed, in the example given, responding for the first 15 min of each daily session depends entirely on conditioned incentive stimuli since primary drug reward does not occur until completion of the first FR10 after15 min has elapsed. The Drd3 full antagonists SB-277011-A in rats (Di Ciano *et al.*, 2003) and PNU 99194-A (5,6-dimethoxy-indan-2-yl dipropylamine) in monkeys (Claytor *et al.*, 2006) dose-dependently decreased responding in the first component of a second-order schedule based on cocaine reward. The full antagonist NGB 2904, on the other hand, produced no significant effects in monkeys (Martelle *et al.*, 2007).

The Drd3 partial agonists BP-897 in rats (Pilla *et al.*, 1999) and CJB 090 [*N*-(4-(4-(2,3-dichlorophenyl)piperazin-1-yl)butyl)-4-(pyridin-2-yl)benzamide] in monkeys (Martelle *et al.*, 2007), like the Drd3 full antagonists SB-277011-A (Di Ciano *et al.*, 2003) and PNU 99194-A (Claytor *et al.*, 2006) dose-dependently decreased responding in the first component of a second-order schedule based on cocaine reward. Like the Drd3 partial agonists BP-897 and RGH-237 (see above), CJB 090 would antagonize the receptor from full activation during conditions of increased DA release associated with the presentation of cocaine-associated cues during drug seeking. The observed effects of these agents are consistent with those of Drd3 full antagonists.

Recently Di Ciano (2008) investigated the effects of local intracerebral injections of SB-277011-A on IV self-administration of cocaine presented according to a second-order FI 15-min (FR10:S) schedule. Injections into the BLA but not the NAc shell or dorsal striatum led to decreased responding. Results implicate Drd3 in the BLA in the control of responding maintained by conditioned stimuli associated with cocaine.

Some researchers have trained animals on similar second-order schedules for food or sucrose reward and similarly evaluated the effects of Drd3 antagonists. Di Ciano *et al.* (2003) concluded that SB-277011-A, at doses that significantly reduced responding that was maintained by conditioned stimuli for cocaine, had no significant effect on responding that was maintained by conditioned stimuli associated with sucrose. However, although the data were somewhat variable and statistical significance was not found, there appeared to be a trend for the highest dose (20 mg/kg) to reduce responding (FIG. 1A). Martelle *et al.* (2007) found that the Drd3 partial agonist CJB 090 significantly reduced responding maintained by conditioned stimuli based on food (FIG. 1C). The full antagonist NGB 2904 had no significant effect but, as was the case for the Di Ciano *et al.* (2003) results, there was a non-significant trend for NGB 2904 to produce a decrease in responding; this was seen in the second but not the first component of a second-order FR5 (FI 6-min) schedule (Martelle *et al.*, 2007) (FIG. 1D). Claytor *et al.* (2006) found that the Drd3 antagonist PNU 99194-A decreased the responding of monkeys for food on a second-order schedule, an effect like that seen with responding for cocaine. Recently, Thanos *et al.* (2008) reported that responding for food presented according to a FR4 schedule was reduced by SB-277011-A but not NGB-2904. Collectively, these results suggest that Drd3 antagonists and partial agonists similarly decrease responding maintained by conditioned incentive stimuli based on cocaine. Drd3 antagonists and partial agonists may decrease responding maintained by conditioned stimuli based on nutritional rewards such as sucrose or food.

Another means to make responding more dependent on conditioned incentive stimuli is to increase the schedule requirement in a simple schedule such as a FR. As mentioned above, Xi *et al.* (2005) found no significant effect of SB-277011-A on cocaine self-administration that was maintained on a FR1 schedule but they observed a significant decrease in responding that was maintained on a FR10. Break points on a progressive ratio (PR) schedule were reduced in animals treated with SB-277011-A or NGB 2904 (Xi *et al.*, 2005; 2006). When nicotine was used as the rewarding stimulus, SB-277011-A similarly decreased break points on a PR schedule (Ross *et al.*, 2007); in that study PR break points for responding for food were not significantly affected by SB-277011-A. These results provide evidence for a differential effect of Drd3 antagonists on responding for drug reward depending on how strongly that responding relies on conditioned incentive stimuli for its maintenance.

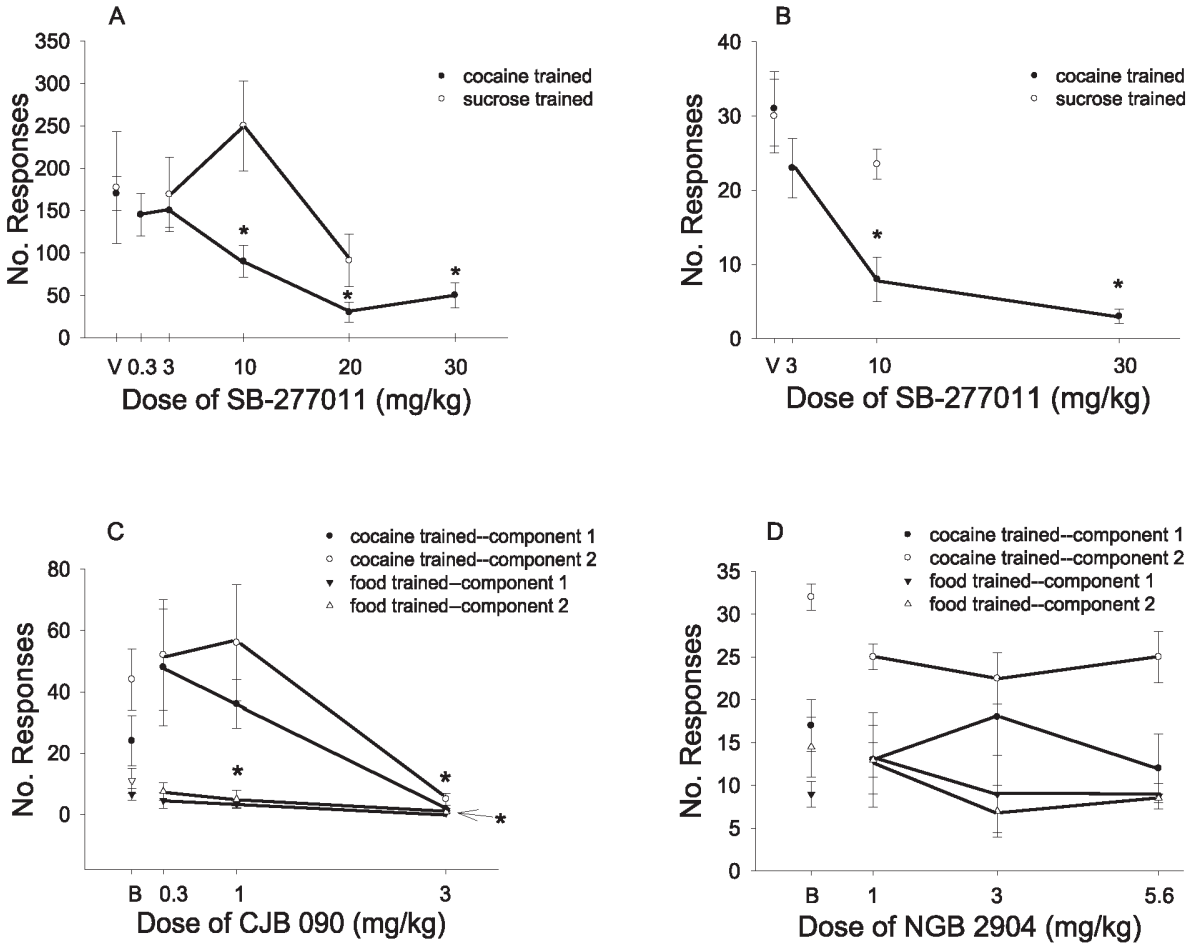

FIGURE 1 Comparison of responding for conditioned cues based on cocaine or nutritional reward. **A**: Mean (±SEM) responding on the first component of a second-order fixed interval 15-min (fixed ratio 10:S) schedule following training with cocaine or sucrose; the dopamine D_3 receptor (Drd3) antagonist SB-277011-A produced a significant decrease in responding for cues associated with cocaine [* *vs* 0 mg/kg (V)] but not sucrose although there was a trend towards less responding for sucrose-related cues with the 20 mg/kg dose of SB-277011-A (adapted from Di Ciano *et al.*, 2003 with permission of the senior author); **B**: Mean (±SEM) responding elicited by a cue associated with cocaine or sucrose after extensive non-rewarded responding. Cocaine cue-induced responding decreased significantly [* *vs* 0 mg/kg (V)] with increasing doses of SB-277011-A while sucrose cue-induced responding did not decrease significantly but there was a trend for less responding with the single dose of SB-277011-A tested (adapted from Cervo *et al.*, 2007 with permission of the senior author and Cambridge University Press: *Int. J. Neuropsychopharmacol.* **10**:167-181, 2007); **C**: Mean (±SEM) responding during the first and second components of a second-order schedule for stimuli based on cocaine or food. The Drd3 partial agonist CJB 090 produced a significant decrease with increasing dose for cocaine-related responding and post hoc tests indicated that the 3.0 mg/kg dose differed significantly from baseline (B) in component 2 (indicated by * above that point); CJB 090 also produced a significant decrease in food-related responding and post hoc tests showed that 1.0 mg/kg significantly decreased responding compared to B in component 2 (indicated by * above that point) and 3.0 mg/kg decreased responding compared to B in components 1 and 2 (* with arrow) (adapted from Martelle *et al.*, 2007 with permission of the corresponding author); **D**: Same method as C but doses of the Drd3 antagonist NGB 2904 produced no significant effect although mean (±SEM) responding for both cocaine- and food-related cues showed a tendency to decrease from baseline (B) at most doses (adapted from Martelle *et al.*, 2007 with permission of the corresponding author).

In summary, the expression of cocaine or nicotine (but not ethanol) self-administration maintained on FR1 or FR2 schedules of reward appears to be minimally affected by treatment with Drd3 antagonists or partial agonists. In contrast, responding maintained by conditioned incentive stimuli based on cocaine appears to be susceptible to the effects of these Drd3 agents. Some of these agents have been found to affect responding maintained by conditioned incentive stimuli based on food or sucrose reward but non-significant effects also have been reported and generally it appears that responding maintained by conditioned incentive stimuli based on drug reward is more affected than that based on nutritional rewards.

Reinstatement studies: The ability of conditioned incentive stimuli to control responding can be studied by using reinstatement procedures (de Wit and Stewart, 1981). After animals are trained to self-administer drug for a number of sessions and responding becomes stable, they can be given multiple sessions without drug reward and without the presentation of conditioned incentive stimuli normally paired with the drug such as a cue light and the sound of the infusion pump. During these non-reward sessions, responding is seen to gradually decline until few responses occur in any one session. At this point, the cues previously associated with drug reward can be presented again to assess their ability to control responding. It has been shown that drug-related cues produce a reinstatement of responding, demonstrating their intact incentive properties (de Wit and Stewart, 1981).

Drd3 antagonists or partial agonists reduce the ability of cocaine-related incentive stimuli to reinstate responding. This has been reported with SB-277011-A, NGB 2904 and BP 897 (Cervo *et al.*, 2003; 2007; Gilbert *et al.*, 2005). SB-277011-A or BP 897 similarly reduced cue-induced reinstatement of responding for oral ethanol (Vengeliene *et al.*, 2006; Heidbreder *et al.*, 2007). The ability of SB-277011-A to attenuate reinstatement of responding for sucrose produced by sucrose-related cues was assessed by Cervo *et al.* (2007) who failed to find a significant effect. However, as was the case in one of the studies using second-order schedules (see above), a limited range of doses was tested and a mild trend was found (FIG. 1B).

Another approach has been to train rats to self-administer cocaine and then to give them a session of non-reward during which cocaine cues are still presented. Results showed that the Drd3 full antagonists SB-277011-A and the partial agonists RGH-237 and BP 897 decreased responding in this paradigm (Gal and Gyertyan, 2006; Gyertyan *et al.*, 2007). In parallel experiments with sucrose or water reward, no effect of SB-277011-A, RGH-237 or BP 897 was found (Gal and Gyertyan, 2006; Gyertyan *et al.*, 2007).

In summary, Drd3 antagonists or partial agonists generally reduced the response-reinstating effects of cues associated with self-administered cocaine or ethanol. These Drd3 agents do not seem to have the same effect on cue-induced reinstatement when the cue is one associated with nutritional reward but this phenomenon so far has received limited research attention and further studies are needed.

Drd3 Antagonists and Conditioned Place Preference (CPP)

In a typical CPP study, the apparatus is a chamber with two distinct compartments connected by a tunnel or an antechamber (see Tzschentke, 1998). The basic procedure involves blocking the connection between the compartments, pairing one chamber with a reward (*e.g.*, 30 min/day for 4 days) and giving equal exposure to the other compartment without the reward. For the test, the connection between the compartments is opened and the amount of time spent in each compartment is assessed. A CPP is established if the group that received the rewarding stimulus in one compartment spends more time there than a control group that did not receive a rewarding stimulus in either compartment (see van der Kooy, 1987). There is a within-subject variant of the conditioning protocol involving a preliminary phase during which the animals are exposed to the two compartments with the connection opened and time in each compartment is recorded. A CPP is indicated by more time in the side paired with the rewarding stimulus on the test day compared to the time spent in that compartment during the preliminary phase (*e.g.*, Spyraki *et al.*, 1982).

CPP can be viewed as an incentive learning task. Accordingly, during the pairing sessions, the stimuli on the side associated with reward acquire an

increased ability to elicit approach and other responses. During the test, animals spend more time there because of this learning (Beninger *et al.*, 1989). The CPP procedure is useful for distinguishing the effects of drugs on acquisition versus expression of learning. Acquisition takes place during the pairing sessions, and expression is evaluated on the test day following conditioning. Experimental drugs can be given during the acquisition phase; if CPP is not observed on the test, the experimental drugs have blocked acquisition. Alternatively, experimental compounds can be given on the test day after conditioning with the rewarding drug alone. If CPP is not observed, the experimental compound has blocked expression of CPP.

The role of Drd3 in acquisition and expression has been evaluated in a number of studies. Results from some studies support a role for Drd3 in expression and not in acquisition of CPP; however, not all results support this conclusion.

The DA transporter blocker cocaine augments synaptic DA and produces a CPP (Morency and Beninger, 1986). The effects of Drd3 antagonists or partial agonists on the acquisition versus expression of CPP based on cocaine are controversial. Vorel *et al.* (2002) and Duarte *et al.* (2003) reported that SB-277011-A and BP 897 blocked *both* the acquisition and expression of a CPP based on cocaine. Alternatively, others have reported no significant effect of the Drd3 agents, SB-277011-A or BP 897 on the *acquisition* of a CPP based on cocaine (Gyertyan and Gal, 2003; Cervo *et al.*, 2005). Gyertyan *et al.* (2007) reported that the novel Drd3 partial agonist RGH-237 at 10 but not 30 mg/kg blocked acquisition of a cocaine CPP but they ascribed no biological importance to this observation. The Drd3 antagonist SB-277011-A blocked expression of a CPP based on cocaine (Vorel *et al.*, 2002; Cervo *et al.*, 2005); the partial agonist BP 897 blocked expression in one study (Duarte *et al.*, 2003) but not in another (Cervo *et al.*, 2005). Thus, a number of (but not all) studies have found that a Drd3 antagonist or partial agonist blocks the *expression* of CPP based on cocaine. When it comes to *acquisition*, there is a split decision: Vorel *et al.* (2002) and Duarte *et al.* (2003) report a significant decrease in CPP when Drd3 antagonists or partial agonists are co-administered with cocaine during conditioning; Gyertyan and Gal (2003), Cervo *et al.* (2005) and Gyertyan *et al.* (2007) report no effect. Thus, Drd3 antagonist or partial agonists often block expression of CPP based on cocaine and sometimes affect acquisition.

The effects of BP 897 on acquisition versus expression of a CPP based on the DA transporter-reversing drug d-amphetamine have been directly compared. Aujla and Beninger (2005) found that BP 897 blocks expression but has no significant effect when given during acquisition. Results support a role of Drd3 in expression, not acquisition of a CPP based on d-amphetamine.

Nicotine produces a CPP that depends on activation of mesolimbic DA neurons (see Le Foll *et al.*, 2005). Few studies have examined the role of Drd3 in nicotine CPP. The Drd3 antagonists SB-277011-A or ST 198 (*N*-(4-[1,2,3,4-tetrahydroisoquinolin-2-yl]-butyl)-3-phenylacrylamide) or the partial agonist BP 897, when injected during the test phase, blocked expression of a CPP based on nicotine (Le Foll *et al.*, 2005; Pak *et al.*, 2006). We know of no studies that have examined the possible role of Drd3 in acquisition of a CPP based on nicotine. Results implicate Drd3 in the expression of nicotine CPP.

The possible role of Drd3 in CPP based on opioid drugs has also been evaluated using the partial agonist BP 897. Expression of a CPP based on morphine was blocked by treatment during the test phase with BP 897 (Frances *et al.*, 2004). In this study, transgenic mice that did not express Drd3 were also tested; they showed a CPP after conditioning with morphine and it was not blocked by BP 897. Duarte *et al.* (2003) reported that BP 897 given either during acquisition or expression of a CPP based on morphine was without significant effect in rats. Vazquez *et al.* (2007) found that BP 897 given during the test phase blocked a CPP based on morphine but only in handled rats, not in a group deprived of maternal contact or in a normally reared group. There is one report of a heroin CPP that was blocked by the Drd3 antagonist SB-277011-A given either during conditioning sessions or during the test session (Ashby *et al.*, 2003). Thus, three of four studies found that full antagonism or partial agonism of Drd3 during the test session for a CPP based on an opioid drug blocked expres-

sion. However, this effect interacted with maternal deprivation and handling. Of the two studies that looked at the effects of a Drd3 antagonist or partial agonist during acquisition on CPP learning, one found a blockade of conditioning based on heroin and the other found no effect on conditioning based on morphine.

When food was used as the rewarding stimulus, BP 897 was without significant effect when given during acquisition or expression (Duarte *et al.*, 2003).

In summary, Drd3 antagonists or partial agonists have consistently been reported to block the expression of a CPP based on cocaine, nicotine, d-amphetamine, morphine or heroin but not food. Studies in which Drd3 antagonists or partial agonists were given during the acquisition phase have produced mixed results. The acquisition of cocaine CPP has been reported to be blocked or unaffected by these Drd3 agents. Results have been consistent in showing no effect of a Drd3 partial agonist on acquisition of CPP based on amphetamine but the two studies available came from the same lab. The effect of Drd3 antagonists or partial agonists on acquisition of a nicotine CPP have not been studied and there is one study showing a block and another showing no effect on acquisition of a CPP based on opioid drugs. Only one study has looked at acquisition of a CPP based on food and it reported no effect of BP 897. Overall, evidence generally supports a role for Drd3 in expression of CPP based on drugs and there are mixed results for the role of Drd3 in acquisition of a CPP based on rewarding drugs. More studies with nutritional rewards (*e.g.*, food) are needed.

Drd3 Antagonists and Conditioned Activity

One of the simplest ways to study incentive learning is with conditioned activity (Beninger, 1983). During pairing trials, animals are given a rewarding stimulus in an experimental environment (*e.g.*, 1 h/day for 3 days). Then, on a test day, they are placed back into that environment and their level of activity is assessed. This group is compared with a control group that has a similar history of exposure to the experimental environment and similar exposure to the rewarding stimulus but the two were never paired. Paired animals show significantly greater activity in the experimental environment (Pickens and Crowder, 1967). This conditioned activity effect can be understood as an example of incentive learning (Beninger, 1983). Thus, reward-associated cues acquire an increased ability to elicit approach and other responses and this manifests as increased activity in the experimental environment on the test day when placed there without reward.

A few studies have evaluated the role of Drd3 in conditioned activity. Using d-amphetamine as the rewarding stimulus, Aujla *et al.* (2002) found that BP 897 given on the test day blocked conditioned activity; when the drug was given on environment-amphetamine pairing days it had no significant effect on the subsequent observation of conditioned activity. In a related study, these researchers evaluated the effects of local injections of BP 897 into the NAc or BLA during acquisition or expression of conditioned activity based on intra-NAc d-amphetamine. Results revealed that infusions of BP 897 into NAc or BLA during test but not during acquisition blocked conditioned activity (Aujla and Beninger, 2004). Thus, BP 897 blocked expression but not acquisition of conditioned activity based on d-amphetamine.

In related studies, Le Foll *et al.* (2002) evaluated the effects of SB-277011-A and BP 897 on the expression of conditioned activity based on cocaine, and Pak *et al.* (2006) evaluated the effects of SB-277011-A on conditioned activity based on nicotine. Both studies found a significant decrease. The effects of the Drd3 antagonist or partial agonist were not assessed on acquisition. Results implicate Drd3 in expression of conditioned activity based on cocaine or nicotine.

Available conditioned activity results implicate Drd3 in the expression of conditioning based on rewarding drugs. The results of the only study so far published suggest that Drd3 is not significantly involved in acquisition. More studies are needed.

Mechanism of Drd3 Involvement in Incentive Learning

The results reviewed here point to a more important role for Drd3 in expression than in acquisition of incentive learning. In the CPP and conditioned activity experiments, results consistently showed that Drd3 antagonists decreased expression of incentive learning. The effects of these agents on

the acquisition of incentive learning were less consistent; although effects were found in some studies, results of many were non-significant. From these results it appears that Drd3 is more involved in the effects of conditioned incentive stimuli (conditioned rewards) on behavior than in the effects of unconditioned incentive stimuli (primary rewards) on behavior.

Results from the self-administration studies can be understood similarly from the point of view of the Drd3 being more importantly involved in the effects of conditioned incentive stimuli on behaviour than in the behavioral effects of unconditioned incentive stimuli. Thus, Drd3 antagonists or partial agonists did not significantly affect self-administration maintained on a FR1 or FR2 schedule but when leaner ratios were used or when a PR schedule was in effect, treatment with a Drd3 antagonist decreased responding. With leaner ratio schedules or PR schedules, responding relies more heavily on conditioned incentive stimuli. Similarly, in second-order schedules where responding is maintained for long periods by conditioned incentive stimuli, Drd3 antagonists decreased responding. Cue-induced reinstatement of responding to self-administer a rewarding drug after a period of non-reward is by definition controlled by a conditioned incentive stimulus and is reliably decreased by Drd3 antagonists. Thus, in CPP, conditioned activity and self-administration studies, Drd3 antagonists or partial agonists decrease the ability of conditioned incentive stimuli to control responding.

Role of Dopamine Drd1-like and Drd2: In considering the possible mechanism, it is useful to review briefly previous suggestions for how DA participates in incentive learning. Thus, stimuli activate hippocampal and neocortical neurons that project to the ventral and dorsal striatum where they stimulate glutamate *N*-methyl-D-aspartate (NMDA) receptors on medium spiny neurons. The influx of calcium ions produced by stimulation of NMDA receptors is thought to create a temporary state of readiness in the dendritic spines that receive these inputs (Miller, 1981; 2008; Miller *et al.*, 1990; Beninger and Miller, 1998). If a rewarding stimulus is encountered right after these stimuli, the DA neurons are phasically activated and DA is released at multiple sites on medium spiny neuron dendritic spines in the striatum. DA acting at D_1-like receptors will stimulate adenylyl cyclase and the cAMP-PKA pathway. The consequences of this DA event will be greatest in spines that are in a state of readiness. It is there that short- and long-term changes are thought to take place leading to incentive learning, the stimuli preceding reward acquiring an increased ability to elicit approach and other responses through their influence on striatal output (Beninger, 1983; Beninger and Gerdjikov, 2004).

An important question is, "What is the substrate of the change that leads to incentive learning?". Drawing on recent findings from many laboratories, Beninger and Gerdjikov (2004) suggested that incentive learning involves a change in the number and phosphorylation state of glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) receptors. In a related paper, we reviewed studies of the role of NMDA and AMPA receptors in the acquisition and expression of incentive learning in a number of reward-related learning paradigms. Results supported a more important role for NMDA receptors in acquisition and AMPA receptors in expression of incentive learning (Beninger and Gerdjikov, 2005).

There is ample evidence for the role of Drd1-like in the acquisition of incentive learning (see Beninger and Miller, 1998). Once incentive learning has taken place, its expression is transiently resistant to the effects of Drd1-like antagonists (*e.g.*, Cervo and Samanin, 1995). The same profile of results has been seen for Drd2 antagonists; they block acquisition and, once acquisition has taken place, incentive learning is transiently resistant to their effects. With repeated testing of a pre-trained response based on reward-related learning, Drd2 antagonists produce a gradual decline in responding (Wise *et al.*, 1978). It remains unknown how Drd1-like and Drd2 work cooperatively to produce and maintain incentive learning.

From the data reviewed in this paper, it appears that Drd3 plays a different role than Drd1-like or Drd2 in incentive learning. On the one hand, Drd1-like and Drd2 are important for the establishment (acquisition) of incentive learning and once incentive learning has taken place its expression is transiently resistant to the effects of Drd1-like or Drd2

antagonists. On the other, Drd3 appears to be important for the expression of incentive learning but less vital for its establishment.

Drd2 vs. Drd3:Some studies have directly compared Drd2 and Drd3 antagonist or partial agonist effects in incentive learning tasks and failed to detect a difference. Thus, the Drd3 partial agonist BP 897 or the Drd2-preferring antagonist raclopride blocked cue-induced reinstatement of responding (Cervo *et al.*, 2003). These results do not support the suggestion that Drd2 and Drd3 play a different role in incentive learning. However, it is important to dissociate effects of drug treatment on the expression of incentive learning versus possible effects on motor ability. Although both BP 897 and raclopride decreased cue-induced response reinstatement on the active lever, examination of the data suggests that raclopride may have had a greater effect on responding on the inactive lever than BP 897, suggesting that the two drugs may have affected motor capacity differentially.

Gal and Gyertyan (2003) reported findings that support a differential role for Drd2 and Drd3 in incentive learning. They trained rats to self-administer cocaine on a FR1 schedule and then tested the effects of SCH 23390 (*R*-(+)-8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3- benzazepine-7-ol), haloperidol and SB-277011-A. The Drd1-like and Drd2 antagonists increased responding whereas the Drd3 antagonist was without significant effect. As was suggested above, self-administration on a FR1 schedule depends relatively less on conditioned incentive cues and is generally unaffected by Drd3 antagonists. This responding is more dependent on the primary rewarding aspect of cocaine and results show that that effect was mitigated by Drd1-like or Drd2 antagonists. Importantly, responding *increased* when animals were treated with these drugs. An increase in responding to self-administer cocaine on a FR1 is normally seen when the concentration of the drug is reduced, so results are consistent with a decrease in amount of reward. The increase also rules out the possibility that the behavioral change was the result of a decrease in motor capacity. Results support a differential role for Drd2 and Drd3 in incentive learning.

In a recent study we directly compared the effects of haloperidol, a Drd2-preferring antagonist to ABT-127 (2,4-di-tert-butyl-6-{4-[3-(4,5-dimethyl-4*H*-[1,2,4]triazol-3-ylsulfanyl)-propyl]-piperazin-1-yl}-pyrimidine hydrochloride), a novel Drd3 antagonist (Drescher *et al.* 2005; Gross *et al.* 2005; Unger *et al.* 2005) on the acquisition and expression of conditioned activity based on cocaine. We first undertook a dose-response study to identify the threshold dose of haloperidol for blocking the acquisition of conditioned activity and the threshold dose of ABT-127 for blocking the expression of conditioned activity. We then used independent groups in a 2 x 2 design with drug and phase (acquisition and expression) as the factors. Results showed a double dissociation with haloperidol blocking acquisition but not expression and ABT-127 blocking expression but not acquisition of conditioned activity based on cocaine (Banasikowski *et al.*, 2007). These results support a differential role for Drd2 and Drd3 in establishment and expression of conditioned incentive learning.

Changes in Drd3: A number of studies have looked for changes in markers for Drd3 induced by rewards. Thus, Drd3 mRNA and, after a delay (16 h), protein was increased in NAc after a single injection of cocaine in rats (Le Foll *et al.*, 2005) and in dorsal striatum following chronic alcohol intake (Vengeliene *et al.*, 2006). In another study, rats with a history of cocaine self-administration were tested for cocaine-induced reinstatement of responding following several hours of non-reward training after withdrawal periods of 1, 7 or 30-31 days. Brains were extracted 24 h later and assayed for Drd3 binding. Results revealed increased Drd3 in ventral regions of the caudate and NAc core in the 30-31 days withdrawal group (Neisewander *et al.*, 2004). In a related experiment, Le Foll *et al.* (2002) injected mice with cocaine or saline and exposed them to a test environment; saline-treated mice (the unpaired group) received cocaine later in the home cage. On the test day, mice were placed into the test environment following a saline injection for a 30-min session, during which conditioned activity was seen in the paired group, and then killed. Results revealed a significant increase in Drd3 (but not Drd1 or Drd2) mRNA and receptors in NAc but not striatum of paired but not unpaired mice. These results showed that changes in Drd3 were specific to mice that had received drug-environment pairings that produced incentive learning manifested as conditioned activity.

These latter results reveal increased numbers of Drd3 in NAc of rats that show incentive learning and the behavioral results reviewed above show that Drd3 antagonists have a greater effect on expression than on acquisition of incentive learning. Thus, Drd3 appears to play a substantially different role in incentive learning than that played by Drd1-like and Drd2. The role played by Drd3 is similar to that played by AMPA receptors (see above); Drd3 seems to be a part of the underlying change that takes place when incentive learning occurs. Unlike Drd1-like and Drd2 that contribute more importantly to establishing incentive learning, Drd3 contributes more importantly to the expression of incentive learning.

CONCLUSION

Drd3 seems to play a more important role in the expression than in the acquisition of incentive learning. This was shown by the results of behavioral studies and supported by molecular studies of Drd3 message and protein. The function of Drd3 seems to be different from that of Drd1-like and Drd2 that have been shown to play a greater role in acquisition than initial expression of incentive learning. The mechanism by which Drd3 contributes to incentive learning is at present an enigma. It will be the task of future studies to delineate the details of the involvement of DA receptors in general and of Drd3 in particular in the brain mechanisms that underlie reward-related incentive learning.

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