Effects of Dopamine D₁ ligands on Eye-blinking in Monkeys:
Efficacy, Antagonism, and D₁/D₂ Interactions

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Abstract

Dopamine D₁ ligands have been classified and ordered according to efficacy in both *in vitro* and *in vivo* studies. In the present experiments, dopamine D₁ ligands reported to differ in *in vitro* efficacy were evaluated for efficacy-related effects on eye blinking in squirrel monkeys. Additional comparisons were made with the effects of D₂ receptor agonists and indirect dopamine agonists. The results show that D₁ agonists increased eye blinking in an efficacy-related manner, whereas the D₁ receptor blocker SCH 39166 only decreased rates of eye blinking. D₁ high-efficacy agonists induced rates of eye blinking that were 2-3 fold greater than observed with dopamine D₂ agonists and indirect agonists. In drug combination experiments, increases in eye blinking induced by the D₁ high-efficacy agonist R-(+)-6- BrAPB were antagonized by both the D₁ antagonist SCH 39166 and the lower efficacy agonist SKF 83959, consistent with dopamine D₁ receptor mediation of these behavioral effects. The dopamine D₂ agonist (+)-PHNO, which selectively activates dopamine D₂ receptors, also attenuated D₁ agonist-induced increase in eye blinking, suggesting that D₂ receptor actions may inhibit D₁-mediated increases in eye blinking. Overall, eye blink rate appears to be a robust behavioral measure that can be used to measure changes in dopaminergic D₁ signaling and as a functional assay of agonist efficacy at dopamine D₁ receptors.

Dopamine D₁ receptor agonists have been ordered according to drug efficacy in both *in vitro* and *in vivo* experiments (see Tables 1 and 2 for summaries of current literature). *In vitro* analyses most often have been based on the extent to which D₁ ligands activate adenylyl cyclase (Kebabian and Calne, 1979) or, more recently, phospholipase C-mediated phosphoinositide (PI) hydrolysis in brain tissue (Undie et al., 1994). While both assays have permitted the identification of differing levels of drug efficacy, it is important to note that results may differ across species or experimental conditions, and that correspondence between *in vitro* and *in vivo* efficacy estimates for D₁ ligands is not uniformly evident. For example, both the D₁ agonists SKF 38393 and SKF 81297 stimulate adenylyl cyclase with low to moderate efficacy in both native striatal and cultured astrocytic tissue from primate species (Vermeulen et al., 1994). In rat brain, however, SKF 38393 continues to display low to moderate D₁ efficacy whereas SKF 81297 produces high, dopamine-like levels of cyclase stimulation (Izenwasser and Katz, 1993; Andersen and Jansen, 1990; Watts et al., 1993; Pifl et al., 1991). Also, the D₁ agonist SKF 83959, which has clear agonist effects in MPTP-lesioned rats and monkeys, fails to stimulate adenylyl cyclase in striatal tissue from untreated rats, resulting in its characterization as an ‘atypical’ D₁ agonist (Gnanalingham et al., 1995, Waddington et al., 1995). However, like SKF 38393, SKF 83959 has good efficacy in stimulating PI turnover, raising the possibility that the two *in vitro* assays may be associated with different physiological or behavioral endpoints (Panchalingam and Undie, 2001; Jin et al., 2003).

Across *in vivo* evaluations of dopamine ligands, efficacy-related differences in D₁-mediated behavioral effects have been more readily observed in mice and monkeys than in rats (see Bergman et al. 2000). Behavioral studies typically indicate that high efficacy D₁ agonists engender more observable behavior than lower-efficacy agonists. For example, Arnt et al. (1992) reported that D₁ agonists with high efficacy for stimulating cyclase activity also produced high levels of locomotion in mice, whereas lesser levels were observed with lower-efficacy agonists including SKF 83959 and SKF75670. In MPTP-induced hemi-parkinsonian monkeys, high efficacy agonists, including A-77636 and SKF 82958, induced contraversive turning, an effect not observed with the D₁ intermediate-efficacy agonist SKF 81297 (Domino and Sheng, 1993). Additionally, D₁ low efficacy agonists, but not high-efficacy agonists, have been shown to produce catalepsy-associated behavior in squirrel monkeys (Rosenweig-Lipson and Bergman, 1994). Although it was not possible to discern an efficacy relationship among drugs in those studies, it is noteworthy that *in vitro* stimulation of adenylate cyclase activity or PI turnover does not preclude the production of *in vivo* antagonist-like effects.

Efficacy-related effects of D₁ agonists in monkeys have been reported in studies of operant performance as well as observable behavior. For example, the extent to which the D₁ receptor blocker SCH 39166 surmountably antagonized the rate-decreasing effects of agonists in studies of schedule-controlled responding of schedule-controlled responding differed among selective D₁ ligands (Bergman et al., 1995). Additionally, compounds considered to have intermediate to high efficacy (e.g., SKF 81297, SKF 82958, and R-(+)-6-BrAPB) were found to support i.v. self-administration behavior in monkeys and to produce cocaine or methamphetamine-like discriminative-stimulus

effects, whereas lower-efficacy agonists (e.g. SKF 38393 and SKF 77434) were not self-administered and antagonized effects of the indirect dopamine agonists in drug-discrimination experiments (Weed and Woolverton, 1995; Weed et al., 1997; Grech et al., 1996; Spealman et al. 1991a; Spealman et al. 1997; Tidey and Bergman, 1998; Sinnott and Nader, 2001). Taken together, differences in the effects of D₁ ligands in these several studies of operant performance have pointed to a functional separation between drugs with intermediate to high efficacy and those with low to no efficacy in *in vitro* studies of cyclase stimulation (Weed and Woolverton, 1997; Bergman et. al., 2000; Tables 1, 2).

The induction of eye blinking is another behavioral effect of dopaminergic agonists that may be amenable to efficacy-based evaluations. Spontaneous eye blinking is associated with central dopaminergic activity and, as documented in dopamine-related pathologies in humans, the disruption of dopamine signaling can alter basal blink rates. For example, Parkinsonian patients have decreased dopamine levels and demonstrated decreased blink rates compared to healthy subjects (Karson et al., 1983; Deuschl and Goddemeier, 1998). In contrast, schizophrenic patients, typically considered to have elevated dopamine levels, have elevated blink rates that can be normalized by treatment with dopamine receptor-blocking neuroleptics (Karson et al., 1981; Karson et al., 1983). Altered blink rates also have been measured in other dopamine-associated CNS disorders such as attention-deficit hyperactivity disorder (Caplan et al., 1996), anxiety and panic disorders (Kojima et al., 2002), and depression (Ebert et al., 1996). However, the extent to which such irregularities involve disruptions in dopaminergic signaling will remain uncertain until the mechanistic bases for these pathologies are better understood.

Consistent with findings in human patients, eye blinking also is reduced in animal models of Parkinson's disease. For example, the severity of Parkinsonian symptoms in MPTP-treated African green monkeys correlated well with the extent to which blink rates decreased in these subjects (Lawrence and Redmond, 1991). Despite clear evidence for dopaminergic involvement in eye blinking, however, few studies have used this behavioral endpoint to investigate the pharmacology of dopamine agonists. Both dopamine D₁ and D₂ receptor agonists can increase eye blinking in vervets and cynomolgus monkeys (Elsworth et al., 1991; Kleven and Koek, 1996) and, of interest, a lower efficacy D₁ agonist, SKF75760, appeared not to increase eye blinking to the same extent as high efficacy D₁ agonists in cynomolgus monkeys (Kleven and Koek, 1996). While suggestive, these initial studies have not been pursued more fully to evaluate the relationship between ligand efficacy and blink rates.

The current research was designed to address this issue by evaluating the effects of dopamine-related ligands on eye blink rates in squirrel monkeys. Squirrel monkeys were used because previous studies provide comparative information regarding other behavioral effects of dopaminergic drugs in this species. First, experiments were conducted to document the effects of D₁ ligands ranging in efficacy from full agonist to antagonist on eye blink rates. The effects of the D₂ agonist (+)-PHNO and the indirect dopamine agonist methamphetamine also were determined. Next, the antagonistic effects of the D₁ receptor blocker SCH 39166 and the D₁ low efficacy agonist SKF 83959 were compared in monkeys treated with the high-efficacy D₁ agonist R(+)-6-BrAPB. Finally, drug interaction experiments were conducted to determine how D₁-mediated alterations in eye blink rate were modified by the selective D₂ agonist (+)-PHNO and D₂ receptor

blocker haloperidol. The results of these studies show that: 1) D₁ agonists increase eye blinking in a dose dependent manner and that the magnitude of increase is dependent on drug efficacy; 2) D₁ antagonists and D₁ low efficacy agonists produce rightward shifts in the dose effect curves of D₁ high efficacy agonists, indicative of D₁ receptor mediation; and 3) co-treatment with the selective D₂ agonist (+)-PHNO appeared to block D₁ agonist-induced stimulation of eyeblinking, suggesting that D₂ receptor activation may inhibit such D₁ receptor-mediated effects.

Methods

Subjects

A group of twelve squirrel monkeys (*Saimiri sciureus*), weighing 750 to 950 g, were housed individually in a climate-controlled vivarium. Monkeys had unlimited access to water and received a daily allotment of high protein monkey chow (Purina Monkey Chow, St. Louis, MO), supplemented with fruit and multi-vitamins. Some monkeys (s323, s484, s60, s98, s152, s62) had previously participated in behavioral experiments and received dopamine agonists and antagonists. Monkeys were weighed daily and their diets were adjusted regularly to maintain constant body weights. The protocol for animal use in these studies was approved by the Institutional Animal Care and Use Committee at McLean Hospital. Subjects in this study were maintained in accordance with guidelines provided by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animals Resources, NIH. This facility is licensed by the U.S. Department of Agriculture.

Apparatus and Experimental Methods

Observational Procedures. Eye blinking was measured during observational experiments that were conducted in a specially constructed chamber. Monkeys sat in a chair with blackened side and back walls to provide color contrast. The front wall of the chair was removed to facilitate videotaping, and a neck plate that maintained a relatively constant orientation of the head was used to facilitate observation of eye blink responses. A curved piece of mirrored Plexiglas also was placed behind the monkey's head to permit viewing of the eye blink response when the monkey turned its head. A compact video

camera (JVC, model GR-AX10), equipped with a telephoto lens, was positioned at a distance of approximately 2 feet in front of the seated monkey. Two vertical fluorescent lights on the sidewalls of the chamber were positioned to provide optimum illumination. The camera image was captured by computer (ATI Multimedia Software, ATI Player) for viewing and recording onto videotape.

In daily control sessions, each monkey was studied for four or five consecutive 15-min components, each consisting of a 10-min habituation period followed by a 5-min period during which eye blinking was videotaped. Test sessions were also comprised of 4 or 5 consecutive components. The video camera displayed the image on a computer screen during the entire video session to allow continual observation of the subject. Observers blind to the treatment conditions later scored the 5-min videotaped session components. An eye blink was defined as a visible, rapid opening and closing of the eyelid. Test components periodically were re-scored by other trained observers to confirm measurement reliability > 90%.

Drug Testing

The direct effects of drugs on eye blinking were studied once or twice a week using cumulative dosing procedures. Briefly, incremental doses of a drug were administered at the outset of the 15-min components of the test session, and the effects of a maximum of 5 cumulative doses could be determined during a single experiment. For most drugs, a complete dose-effect curve was established in one session; however, for some drugs, a full dose effect curve could only be obtained by testing two or three overlapping dose

ranges. Each drug was studied in four or more subjects, and the order of drug testing varied among individuals.

Drug combination studies were conducted in groups of three or four monkeys (see Table 1). For antagonism studies, each of several doses of either SCH 39166 (0.1 – 1.0 mg/kg) or SKF 83959 (0.1 – 3.0 mg/kg) was administered i.m. 15 min before test sessions in which the effects of cumulative doses of R-(+)-6-BrAPB were determined in successive components. In experiments to evaluate D₁-D₂ interactions, each of several i.m. doses of haloperidol (0.1 – 1.0 mg/kg) or a single dose of (+)-PHNO, 0.003 mg/kg, was administered 15 min before test sessions in which the effects of cumulative doses of SKF 82958 were determined. Observations and videotaping were conducted in the manner previously described. For time course studies, a single injection of SKF 82958 (0.1 or 1.0 mg/kg) was administered i.m. prior to videotaping 5-min periods at 5, 10, 15, 30, 45, 60, and 120 min post-injection.

Drugs

Methamphetamine, SKF 82958 HBr [(±)-6-chloro-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide], SKF 38393 [(±)-1-phenyl-2,3,4,5-tetrahydro-(1H)-3-benzazepine-7,8-diol hydrochloride], SKF 77434 [(±)-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide], SKF 81297 [(±)-6-chloro-7,8-dihydroxy-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide], and R-(+)-6-BrAPB [R-(+)-6-bromo-7,8-dihydroxy-3-allyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide] were purchased from Research Biochemicals International (Sigma-Aldrich). SKF 83959 HBr [6-chloro-7,8-dihydroxy-3-methyl-1-(3-

methylphenyl)-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide] was supplied by Research Biochemicals International (Natick, MA) through the Chemical Synthesis Program of the National Institute of Mental Health (Contract N01MH30003). SCH 39166 [(-)-*trans*-6,7,7 α ,8,9,13 β -hexahydro-3-chloro-2-hydroxy-*N*-methyl-5*H*-benzo[*d*]naphtho[2,1-*b*]azepine] and (+)-PHNO [(+)-*N*-propyl-hydroxynaphoxazine] were graciously supplied by Schering-Plough, Inc. and Merck, Sharpe, and Dohme, respectively. Except for the benzazepines, all drugs were prepared in sterile water, and warmed and/or sonicated prior to administration as necessary. Stock solutions of the benzazepines were prepared in a solution of 0.1% ascorbic acid in sterile water prior to further dilution. All drugs were administered by intramuscular (i.m.) injection in volumes of approximately 0.2-0.4 ml.

Data Analysis

Eye blink rates were computed as total number of eye blinks divided by the duration of time that the subject's eyes were visible during the 5-min test component (usually >290 seconds). Vehicle injections did not affect rates of eye blinking during the four sequential components of vehicle-control sessions; therefore, mean control values for rates of eye blinking (mean $\pm$ S.E.M.) were calculated by averaging blink rates across vehicle-control sessions. For individual monkeys, data were expressed as the rate of eye blinking effect after each cumulative dose (blinks per minute). The effects of vehicle and each drug were averaged for each group of monkeys to express mean $\pm$ S.E.M. values. The effects of a dose of drug were considered to be statistically significant when the

mean value for that dose lay outside the 99% confidence interval for mean control rates of eye blinking in the same group of monkeys.

Results

Control rates of eyeblinking

Control values for eye blink rates never exceeded 20 blinks per minute in any subject and, for the group of 12 monkeys, averaged 9.2 ± 1.4 blinks per minute. Control values for different groups of subjects in which drugs were studied ranged from 5.5 ± 0.3 blinks per minute to 13.2 ± 2.2 blinks per minute. Table 3 shows that control values varied somewhat across monkeys, but were relatively stable across control sessions within individual subjects. Factors that contributed to individual differences in blink rate were not identified, and monkeys were not grouped for drug testing on the basis of control values. However, monkeys with higher blink rates were fully mature and had participated in extended studies of the behavioral effects of dopaminergic ligands, whereas monkeys with lower blink rates were relatively young and had not previously participated in studies involving drug treatment.

Effects of dopamine D₁ ligands

The dopamine D₁ agonist R-(+)-6-BrAPB produced the highest rates of eye blinking, demonstrating a 9-fold increase over control values at the highest dose tested, 0.3 mg/kg (Fig. 1a). The dopamine D₁ agonists SKF 81297 and SKF 82958 were comparably potent and produced high rates of eye blinking, yielding 7- to 8-fold increases over control values, respectively (Fig. 1a). A time course evaluation of the highest dose of SKF 82958, 1.0 mg/kg, showed that rates of eye blinking increased with a rapid onset of action that dissipated in an exponential manner over the course of 60 min (Fig. 2). The lower dose of SKF 82958, 0.1 mg/kg, produced small increases in rates of

eye blinking initially after injection, and these elevated rates completely returned to baseline values by 45 min.

The D₁ agonists SKF 83959 and SKF 77434 also generally increased rates of eye blinking, although to a lesser extent than observed with SKF 82958, SKF 81297, and R-(+)-6-BrAPB (Fig. 1b). Cumulative doses of SKF 83959 resulted in rates of eye blinking that averaged approximately 4-fold above control values and appeared to plateau at this level. SKF 77434, at 3.0 and 10.0 mg/kg, also increased rates of eye blinking 3.5-fold above control values. The higher dose of 17.8 mg/kg SKF 77434 either did not further increase eye blink rates or, in some monkeys, decreased blink rates toward control values. The agonist SKF 38393 produced small, if any, increases in rates of eye blinking (Fig. 1b). The largest effect observed with SKF 38393 was a 1.5-fold increase over control values with the lowest dose tested (3.0 mg/kg). By contrast, the highest dose of SKF 38393, 30 mg/kg, decreased rates of eye blinking to 50% of control values.

In contrast to the increases in rates of eye blinking produced by most D₁ agonists, the D₁ receptor blocker SCH 39166 only decreased rates of eye blinking (Fig. 1b). Low doses, 0.01-0.03 mg/kg, slightly decreased blink rates to 84 and 77% of control values, respectively. The larger dose of 0.1 mg/kg SCH 39166 decreased rates of eye blinking to an average of 2 blinks per minute, or approximately 12% of control blink rates.

The maximum increase in eye blink rates is plotted for each dopamine D₁ ligand in Figure 3. As shown, R-(+)-6-BrAPB produced the largest increase above vehicle, followed by SKF 82958, SKF 81297, SKF 83959, and SKF 77434. The remaining two ligands SKF 38393 and SCH 39166 did not stimulate eye blink rates significantly above vehicle values.

Effects of indirect and D₂ dopamine agonists

The indirect dopamine agonist methamphetamine produced dose-dependent increases in rates of eye blinking (Fig. 1c). At a dose of 1.0 mg/kg, methamphetamine increased blink rate to 4-fold above control values. A higher dose of methamphetamine, 3.2 mg/kg, did not appear to increase eye blink rates further; however, this higher dose of methamphetamine produced agitation and other behavioral effects that precluded accurate scoring of blink rates and, therefore, was tested in one subject only. The dopamine D₂ agonists, quinolorane and (+) PHNO, produced small increases in rates of eye to approximately 20 blinks per min (Fig. 1c).

Drug interaction studies

Several doses of SCH 39166 were administered prior to cumulative dose effect determinations of R-(+)-6-BrAPB-induced eye blinking. SCH 39166 antagonized the effects of the D₁ agonist R-(+)-6-BrAPB on eye blinking and shifted the dose effect function rightward and in a parallel manner (Fig. 4). Each half-log unit increase in SCH 39166 shifted the R-(+)-6-BrAPB dose effect curve approximately 3-fold to the right. A Schild plot analysis of the antagonist effects of SCH 39166 demonstrated a pA₂ value of 7.12 with a slope of -1.22 ± 0.23 .

In further experiments, increasing doses of SKF 83959 also were administered prior to R-(+)-6-BrAPB. Like SCH 39166, SKF 83959 generally shifted the dose effect curve for R-(+)-6-BrAPB-induced eye blinking to the right (Fig. 5). Pretreatment with the low dose of SKF 83959, 0.1 mg/kg, did not shift the entire dose effect curve, but

attenuated the effects of the highest dose of R-(+)-6-BrAPB. The intermediate doses of SKF 83959, 0.3 and 1.0 mg/kg, produced approximate 3-fold shifts in the R-(+)-6-BrAPB dose effect curve, and the highest dose, 3.0 mg/kg SKF 83959, shifted the curve approximately 30-fold to the right.

The dopamine D₂ antagonist haloperidol was administered prior to cumulative doses of the D₁ full agonist SKF 82958 and did not appear to antagonize the effects of SKF 82958 in a surmountable manner (Fig. 6). Thus, whereas relatively high doses of haloperidol may have partially blocked the effects of 1.0 mg/kg SKF 82958, the effects of haloperidol were neither dose-related nor robust ($\leq$ 3-fold change following the high dose of 1.0 mg/kg).

The dopamine D₂ agonist (+)-PHNO was administered alone or prior to cumulative doses of the D₁ full agonist SKF 82958 (Fig. 7). The dose of 0.003 mg/kg (+)-PHNO increased eye blinking to 27 blinks per min. When administered as a pretreatment to SKF 82958, 0.003 mg/kg (+)-PHNO increased eye blinking above the effects of the low dose of SKF 82958 alone (0.03 mg/kg) and decreased eye blinking to below effects of the higher doses of SKF 82958 alone (0.1 and 1.0 mg/kg).

Discussion

R-(+)-6-BrAPB, SKF 82958, and SKF 81297 were the most efficacious compounds, leading to rates of eye blinking that were 7- to 9-fold above control values. SKF 82958 and SKF 81297 were comparably potent, but R-(+)-6-BrAPB was slightly more potent than these two D₁ high efficacy agonists. SKF 77434 and SKF 83959 demonstrated lower efficacy agonist activity, increasing eye blinking only 3-fold above control values. Although these D₁ partial agonists produced similar increases in eye blinking, SKF 83959 was 100 times more potent than SKF 77434. The highly selective dopamine D₁ ligand SKF 38393 was a weak agonist in these studies, producing very small increases in eye blinking at the doses tested. Using eye blinking measurements, these dopamine D₁ agonists displayed a rank order of efficacy: R-(+)-6-BrAPB > SKF 82958 ≥ SKF 81297 > SKF 83959 = SKF 77434 > SKF 38393. These data are consistent with some previous efficacy classifications of dopamine D₁ agonists. Interestingly, previous work demonstrated that SKF 81297 demonstrated low efficacy for increasing adenylate cyclase activity in squirrel monkey striatal tissue (Izenwasser and Katz, 1993); however, this compound produces behavioral effects similar to those of high efficacy agonists in the present study as well as in other behavioral studies in monkeys (Spealman et al., 1997; Weed et al., 1997; Tidey and Bergman, 1998). Overall, these data demonstrate that D₁ agonists can elicit a high degree of eye blinking activity. The large range of effect that can be observed suggest that measurement of eye blinking can be a useful indicator of dopamine D₁ agonist efficacy in primate species.

The effects of D₁ agonists on eye blinking appear to be mediated solely by D₁ receptor activation. The selective dopamine D₁ antagonist SCH 39166 produced parallel shifts in the R-(+)-6-BrAPB dose effect, consistent with mediation by the dopamine D₁ receptor. In this regard, pA₂ analysis of the shift in the R-(+)-6-BrAPB dose effect curve reported a slope of 1.22 ($\pm$ 0.23) and a pA₂ value of 7.12, supporting the view of competitive antagonism at the D₁ dopamine receptor. Moreover, the D₂ dopamine receptor antagonist haloperidol did not reliably antagonize the effects of the D₁ agonist SKF 82958, consistent with a predominant role for the D₁ receptor in D₁ agonist-induced changes in eye blinking.

The D₁ low efficacy agonist SKF 83959 produced moderate increases in eye blinking and produced rightward shifts in the dose effect curve for the D₁ full agonist R-(+)-6-BrAPB. These data demonstrate that SKF 83959 is a partial agonist at the dopamine D₁ receptor and, as with the behavioral effects of other pharmacological classes, the partial agonists in the D₁ receptor system can act as competitive antagonists.

Dopamine D₂ receptor activation with the D₂ agonists, (+)PHNO and quinelorane, only produced small increases in eye blinking. However, D₂ receptor activation with (+)-PHNO also antagonized D₁ agonist-induced increases in eye blinking at high doses, similar to the effects observed with low efficacy D₁ agonists. Based on previous findings, it is unlikely that (+)-PHNO is activating the D₁ receptor at the doses used in this study (Rosenzweig-Lipson et al., 1994; Bergman et al., 1995; Domino and Sheng, 1993; Elsworth et al., 1991). More likely, D₂ receptor activation by (+)-PHNO may have inhibited D₁ agonist-induced eye blinking in these experiments. D₁/D₂ agonist interactions have received some attention in previous behavioral studies. In keeping with

earlier suggestions of facilitative or synergistic interactive effects of D₁ and D₂ receptor activation (Walters et al. 1987), co-administration of D₁ and D₂ agonists has been reported to produce exaggerated contralateral turning in 6-OHDA-lesioned rats (Gnanalingham et al., 1995). However, in the same study, this synergistic effect was not observed in MPTP-treated monkeys. Likewise, combined activation of D₁ and D₂ receptors has not been found to potentiate the effects of either compound in either methamphetamine-discrimination studies in rats (Nielsen et al., 1989) or cocaine-discrimination studies in monkeys (Spealman et al., 1991b). The present findings provide further evidence that D₁ and D₂ receptor activation is not necessarily additive or synergistic and, moreover, that D₂ receptor activation can moderate behavioral effects of dopamine D₁ receptor activation. In this regard, it may be noteworthy that, in the present study, the indirect dopamine agonist methamphetamine only increased eye blinking 4- to 6-fold above control values. Possibly, the limited effects of methamphetamine on eye blinking are indicative of moderating effects resulting from its indirect agonist activation of dopamine D₂ receptors.

Interestingly, the D₁ antagonist SCH 39166 nearly eliminated eye blinking (88% decrease) at the highest doses tested. The state of dopamine D₁ receptors *in vivo* is not yet fully elucidated, leaving the mechanism that mediates these effects open to speculation. It may be that dopaminergic tone regulates normal levels of eye blinking and, thus, that blocking D₁ receptors decreases dopamine-mediated signaling and inhibits eye blinking. Similar explanations can be used to explain decreased eye blinking in human Parkinson's patients, in which decreased dopaminergic activity has been related to decreases in eye blink rate. On the other hand, the dopamine receptor may be

constitutively active. In this case, SCH 39166 may have inverse agonist activity that could functionally decrease D₁ receptor-G protein coupling (Cai et al., 1999; Martin et al., 2001), leading to the same behavioral outcome. From this perspective, compounds that previously have been classified as neutral antagonists but have inverse agonist activity would be anticipated to have effects *in vivo* – in addition to their antagonist action. Along these lines, dopamine D₁ antagonists including SCH 39166 can have behavioral effects comparable to those of low efficacy D₁ agonists (Rosenzweig-Lipson and Bergman, 1994). These observations may be more consistent with drug-induced decreases in D₁ receptor activation than inverse agonist activity produced by D₁ receptor blockers.

In conclusion, the present data demonstrate that eye blink measurement can be used to identify dopamine D₁ agonists differing in agonist efficacy and to distinguish between different types (i.e., D₁, D₂) of dopaminergic actions. Additionally, the present studies provide an interesting insight into D₁:D₂ receptor interactions, suggesting that D₂ receptor activation may moderate some D₁ agonist-induced behavioral effects in a primate species. These data provide a basis for using eye blinking as an assay with which to identify and classify novel dopamine D₁ agonists as well as to further investigate the behavioral consequences of dopamine D₁ and D₂ receptor-mediated interactions.

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Footnotes

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Figure 1. The effects of dopamine D₁ ligands on eye blinking in squirrel monkeys:
Abscissae: cumulative i.m. doses of drugs; ordinates: rates of eye blinking (blinks per minute). Symbols above Veh shows mean $\pm$ 99% CI for the effects of vehicle injections for Groups 1-3 (Table 1). Data in the top panel (a) were obtained in Groups 1 and 3 (SKF 81297 and R-(+)-6-BrAPB) or Groups 2 and 3 (SKF 82958); data in the middle panel (b) were obtained in Group 1 and additionally, for SKF 38393 and SKF 83959, Group 3; data in the lower panel (c) were obtained in Group 1 or, for quinelorane, Group 3. Results are presented as the mean ($\pm$ sem) for rates of eye blinking.

Figure 2. Time course of SKF 82958-induced increase in eye blinking. Abscissa: time following i.m. injection; ordinate: rates of eye blinking. Symbol above Veh shows mean $\pm$ 99% CI for the effects of vehicle injections in the 3 Group 2 subjects in which data were obtained.

Figure 3. Maximum effects of D₁ ligands on eye blink rate. Abscissa: Dopamine D₁ ligands studied in the present experiments; ordinate: rate of eyeblinking following the most effective dose of each drug (taken from Figure 1). Ligands are listed in descending order of functional efficacy: R-(+)-6-BrAPB, SKF 82958, SKF 81297, SKF 83959, SKF 77434, SKF 38393, and SCH 39166. Bar above Vehicle shows effects of vehicle injection averaged across all groups.

Figure 4. The effects of pretreatment with different doses of the D₁ antagonist SCH 39166 on R-(+)-6-BrAPB-induced eye blinking. Data are presented as the mean effects ($\pm$ 99% CI) of drug treatment in Group 1 subjects (see Table 1). Schild plot analysis of the data yields a slope of -1.22 ($\pm$ 0.23) and a pA₂ value of 7.12. Other details as in Figure 1.

Figure 5. The effects of pretreatment with different doses of the D₁ partial agonist SKF 83959 on R-(+)-6-BrAPB-induced eye blinking. Data are presented as the mean effects ($\pm$ 99% CI) of drug treatment in Group 3 subjects (see Table 1). Other details as in Figure 1.

Figure 6. The effects of pretreatment with haloperidol on SKF 82958-induced eye blinking. Data are presented as the mean effects ($\pm$ 99% CI) of drug treatment in four subjects Ss2, Ss9, Ss33, SS41. Other details as in Figure 1.

Figure 7. The effect of pretreatment with 0.003 mg/kg (+)-PHNO on SKF 82958-induced eye blinking. Data are presented as the mean effects ($\pm$ 99% CI) of drug treatment in Group 3 subjects (see Table 1). Other details as in Figure 1.

Table 1. Comparison of *in vitro* determinations of D₁ efficacy in different assay systems and species.

Unless otherwise noted, data for each ligand were directly compared to effects of dopamine (DA) in same study.

Reference	<i>In vitro</i> assay	Tissue / Cell	Efficacy estimate		
			High (>70% of DA)	Intermediate (30-70% of DA)	Low (<30% of DA)
Andersen and Jansen (1990)	adenylyl cyclase (striatum)	rat	dopamine SKF81297	SKF38393 SKF77434	SKF75670 apomorphine
Piffl et al. (1991)	adenylyl cyclase (striatum)	rat ¹	dopamine	SKF38393	
		rhesus monkey ¹	dopamine		SKF38393
Arnt et al. (1992)	adenylyl cyclase (striatal homogenates)	rat	dopamine SKF80723	SKF75670 SKF38393 SKF81297	SKF83959
Izenwassser and Katz (1993)	adenylyl cyclase (caudate-putamen)	rat	dopamine SKF82958 6-Br-APB SKF81297	dihydraxidine SKF38393	SKF75670
		squirrel monkey	dopamine SKF82958	dihydraxidine SKF38393	6-Br-APB SKF81297 SKF75670
Watts et al. (1993)	adenylyl cyclase (striatum)	rat	dopamine dihydraxidine	SKF38393	
		monkey	dopamine dihydraxidine	SKF38393	
Vermeulen et al. (1994)	adenylyl cyclase (cultured astrocytes)	rat	dopamine SKF81297	SKF38393	
		rhesus monkey	dopamine	SKF81297	SKF38393
		human	dopamine A68930	SKF81297	SKF38393
Panchalingham and Undie (2001)	PI hydrolysis ² (brain slice)	monkey	dopamine SKF38393 SKF83959		
Jin et al. (2003)	PI hydrolysis ²	PC12 cells with D1 DAR			SKF38393 SKF83959
	PI hydrolysis ² (frontal cortex)	rat	SKF83959	SKF81297 SKF38393	
	adenylyl cyclase (striatum)	rat	dopamine SKF81297	SKF38393	SKF83959
		D1A/PC12 cells	dopamine SKF81297	SKF38393	SKF83959

¹ raw values of pmol/mg protein/min recalculated to percent DA, ² not compared to DA

Table 2. Comparison of *in vivo* estimates of D₁ efficacy in different behavioral assays and species.

Reference	<i>In vivo</i> assay	Subjects	Efficacy estimate		
			High	Intermediate	Low
Arnt et al. (1992)	turning behavior ¹	6-OHDA lesioned rats	SKF83959 SKF75670 SKF38393 SKF81297		
	locomotor activity ²	mice	SKF81297	SKF75670	SKF83959
Gnanalingham et al. (1995)	turning behavior	6-OHDA lesioned rats	SKF83959	SKF75670	SKF38393 SKF82958
Domino and Sheng (1993)	turning behavior	MPTP-treated pigtail macaques	SKF82958 SKF81297 A77636 l-Dopa		
Rosenzweig-Lipson and Bergman (1994)	Catalepsy ³	squirrel monkeys	SKF81297 SKF82958		SKF38393 SKF76570
Weed et al. (1997)	self-administration ⁴	rhesus monkeys	SKF82958 R(+)-6-BrAPB	SKF81297	
Weed and Woolverton (1995)	self-administration ⁴	rhesus monkeys	SKF81297 SKF82958 R(+)-6-BrAPB		SKF38393 SKF77434
Grech et al. (1996)	self-administration ⁴	squirrel monkeys	SKF82958	SKF81297	SKF77434
Spealman (1991)	cocaine discrimination	squirrel monkeys	SKF82958 SKF81297		
Sinnott and Nader (2001)	cocaine discrimination	rhesus monkeys	SKF81297		SKF38393
Tidey and Bergman (1998)	methamphetamine discrimination ⁵	squirrel monkeys	SKF81297 SKF82958 dihydroxidine		SKF77434
Kleven and Koek (1996)	blink rate	cynomolgus monkeys	SKF81297 SKF82958		SKF75760

¹ percentage of apomorphine response; ² percentage of methylphenidate response; ³ SCH 39166 was used for comparison; ⁴ subjects trained with cocaine

Table 3. Control rates of eye blinking. Values show data for individual subjects collapsed across session components and averaged over four or more vehicle-injection sessions. Drugs that were studied in each group are given in figure legends.

Group 1		Group 2		Group 3	
Monkey	Blinks/min ($\pm$ sem)	Monkey	Blinks/min ($\pm$ sem)	Monkey	Blinks/min ($\pm$ sem)
s323	12.92 (0.89)	s2	7.58 (0.78)	s9	5.48 (0.84)
s484	16.31 (0.89)	s3	5.15 (0.28)	s33	5.92 (0.48)
s60	17.37 (1.31)	s7	3.90 (0.42)	s41	5.71 (0.21)
s98	6.35 (1.13)	s9	4.18 (0.49)		
		s62	11.89 (0.60)		
		s152	11.86 (1.69)