Postcontest Blockade of Dopamine Receptors Inhibits Development of the Winner Effect in the California Mouse (Peromyscus californicus)

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The winner effect is an accumulation of previous wins that increase future winning. A primary unanswered question about the winner effect is how do individuals integrate information about previous wins? Dopamine (DA) has been implicated because phosphorylated tyrosine hydroxylase (pTH), the rate-limiting enzyme for DA biosynthesis, is elevated following multiple winning experiences. Moreover, DA receptor blockers and agonists influence aggression when administered prior to male-male contests. In the current study, we administered D1- and D2-like DA receptor antagonists immediately after a contest and examined the development of the winner effect in the territorial California mouse, Peromyscus californicus. During a 3-contest training phase, resident males experienced winning contests, followed immediately by a peripheral injection of either a DA receptor antagonist or vehicle or a handling experience (without injection). The DA receptor antagonists used in this study did not influence locomotion. To assess the cumulative effects of previous winning, males were subjected to a final test contest with a more competitive intruder. The winner effect was significantly decreased by both D1- and D2-like receptor antagonists administered during training. During the test contest, attack behavior was significantly reduced by previous administration of both types of DA receptor antagonists compared with controls. D1-like receptor blockade also diminished chasing behavior, whereas D2-antagonist treated animals continued to pursue opponents. During training against a less competitive intruder, there was no difference in aggressive behaviors between experimental and controls males. Our data indicate that DA activity between contests is concomitant with the competitive advantage gained from multiple winning experiences.

Keywords: competition, antagonistic behavior, winner effect, Peromyscus californicus, dopamine, mice

Agonistic behavior is evolutionarily conserved because it is important for gaining and maintaining access to resources such as food and mates. In reality, competitors are rarely evenly matched, and contest outcomes are often determined by residency status and ownership of resources (Fuxjager, Montgomery, Becker, & Marler, 2010). When all else remains equal, winners are determined by the resource holding potential/resource value model, which takes both fighting ability and value of the contested resource into account (Parker & Rubenstein, 1981). Agonistic behavior is energetically costly and can lead to decreased survivorship (Duffy, 1989; Marler & Moore, 1989; Silverman & Dunbar, 1980). As a consequence, males must weigh the relative costs and benefits before engaging an opponent, which indicates a high degree of behavioral and motivational plasticity and an assimilation of relevant information. Winning can also be determined by previous contest outcomes. The probability of winning an aggressive contest is appreciably increased after each previous victory (Oyegbile & Marler, 2005). Demonstration of these “winner effects” (Dugatkin, 1997; Hsu, Earley, & Wolf, 2006) ranges across mammals (Oyegbile & Marler, 2005; Schwartzer et al., 2013), birds (Drummond & Canales, 1998), fish (Beaugrand, Goulet, & Payette, 1991; Chase, Bartolomeo, & Dugatkin, 1994), and invertebrates (Hoefler, 2002; Whitehouse, 1997). The cumulative nature of the effects of previous victories on the probability of winning suggests a neurobiological integration of the social experiences between contests. Increases in postcontest testosterone (T; Fuxjager, Oyegbile, & Marler, 2011; Oyegbile & Marler, 2005) also influence the probability of winning. Androgens (Fuxjager et al., 2011; Oyegbile & Marler, 2005; Trainor et al., 2004) and androgen receptors (Fuxjager et al., 2010) have been the primary focus of recent research examining the winner effect. Bearing in mind the plasticity in the expression of aggressive behavior, it is likely that there are other contributing neurobiological mechanisms. For example, steroid hormones may exert indirect actions on behavior through changes in neurotransmitter synthesis. One likely candidate in the formation of the winner effect is the neurotransmitter dopamine (DA) because T administration elevates DA levels in the brain (Hull, Du, Lorrain, & Matuszewich, 1995) and DA receptors...
become more androgen sensitive following anabolic-androgen steroid (AAS) treatment (Birgner et al., 2008; Kindlundh, Lindblom, Bergström, & Nyberg, 2003).

To date, much research indicates an important role of DA in the immediate regulation of competitive behavior (Bondar & Kudraytseva, 2005; de Almeida, Ferrari, Parmigiani, & Miczek, 2005; Ferrari, van Erp, Tornatzky, & Miczek, 2003; Kudraytseva, Lipina, & Koryakina, 1999; May & Kennedy, 2009; Puglis-Allegre & Cabib, 1988; Ricci, Schwartzter, & Melloni, 2009; Schwartzter & Melloni, 2010; Sokolov & Cadet, 2006; van Erp & Miczek, 2000). DA receptor blockade, as well as DA receptor knockout, diminishes (Drago, Padungchaichot, Accili, & Fuchs, 1998; Kudraytseva et al., 1999; Miczek, Maxson, Fish, & Facidomo, 2001; Rodríguez-Arias, Miñarro, Aguilar, Pinazo, & Simón, 1998; Sánchez, Amf, Hyttel, & Moltzen, 1993; Sokolov & Cadet, 2006), while DA receptor agonists facilitate aggressive behavior (Dennis & Cheng, 2011; Lammers & van Rossum, 1968; Nikulina & Kapralova, 1992). DA can also be released in anticipation of (Ferrari et al., 2003) and in response to agonistic contests (van Erp & Miczek, 2006; van Erp & Miczek, 2007). Moreover, DA is associated with androgens, a key mediator of the winner effect (Fuxjager et al., 2010; Fuxjager et al., 2011; Trainor, Bird, & Marler, 2004). For example, rats treated daily for two weeks with nandrolone decanoate (AAS) exhibit elevated D1-receptor mRNA expression in the amygdala and nucleus accumbens (Birgner et al., 2008). Furthermore, animals treated with AASs during adolescence experience elevated levels of both tyrosine hydroxylase (a marker of DA production) within regions of the hypothalamus and aggression later in life (Ricci, Schwartzter, & Melloni, 2009; Schwartzter & Melloni, 2010). One hypothesis is that DA activity increases significantly in response to repeated exposure to T (Fuxjager et al., 2010; Oyegbile & Marler, 2005) among animals with multiple winning experiences (Schwartzer et al., 2013); increases in both winning and expression of tyrosine hydroxylase (a marker of DA production) within regions of the social brain network were observed among male hamsters following repeated winning contests (Schwartzer, Ricci, & Melloni, 2013). Together, these studies indicate that DA is a potent mediator of aggressive behavior and implicate DA in the winner effect, although whether the increased ability to win future male–male contests occurs via DA still needs to be directly tested through manipulations of DA receptor sensitivity.

In the current study, we tested whether DA is involved in the accumulation and integration of information gathered from each winning contest. Because traditional pharmacological manipulations of DA have consequences on both mesocorticolimbic (“reward”) and nigrostriatal (“motor”) systems, previous studies on DA’s involvement in the regulation of aggression have been confounded by motor deficits (e.g., Bondar & Kudraytseva, 2005; Jerrell, Hwang, & Livingston, 2008; Nikulina & Kapralova, 1992; Rodríguez-Arias et al., 1998). Therefore, we used doses that lack effects on locomotor behavior and administered DA receptor antagonists after each, but well before, the subsequent winning contest during the training phase to test effects on future behavior. Blockers administered prior to a training winning experience simply decrease the levels of aggression during that particular contest as stated earlier.

DA receptors are classified into two types: D1-like receptors (D1 and D5) and D2-like receptors (D2, D3, and D4; Sibley, Monsma, & Shen, 1993), and they are often characterized by both their opposing physiological effects (Sibley et al., 1993) and distribution in the brain, which may reflect distinct functional roles (Missale et al., 1998). Activation of D1-like receptors results in neural excitation (Monsma, Mahan, McVittie, Gerfen, & Sibley, 1990), whereas D2-like activation decreases neuronal activity (Bunzow et al., 1988). Both D1- and D2-like receptor subtypes are implicated in the regulation of aggressive behavior in numerous studies (Aguilar, Miñarro, Pérez-Iranzo, & Simón, 1994; Beiderbeck et al., 2012; Bondar & Kudraytseva, 2005; Dennis, Muir, & Cheng, 2006; Sánchez et al., 1993; Schwartzter & Melloni, 2010). The D1-like receptor antagonist SCH-23390 reduces offensive aggression (Bondar & Kudraytseva, 2005; Sánchez et al., 1993), as does administration of the D2 antagonists, emapralide (Sánchez et al., 1993; Schwartzter & Melloni, 2010) and raclopride (Aguilar et al., 1994; Dennis et al., 2006). Local microinjection studies, however, indicate that D2-like receptors may influence aggression directly, whereas D1-like receptors have indirect effects via suppression in mobility and general social interest (Schwartzer & Melloni, 2010). In addition, studies indicate that D2-like receptors may also regulate aggression through the control of defensive behaviors (Puglisi-Allegra & Cabib, 1988; Swedan, Edinger, & Siegel, 1991). In cats, injection with a D2-like receptor agonist increased defensive hissing whereas treatment with a D2-like receptor antagonist decreased this behavior; in contrast, treatment with D1-like agonists and antagonists did not influence this behavior (Swedan et al., 1991). Although our current understanding is still nebulous given the opposing responses of these two receptor families, it is likely that the receptor types regulate aggression through distinct mechanisms (Bondar & Kudraytseva, 2005; Rodriguez-Arias et al., 1998; Tidey & Miczek, 1992).

The aggressive (Bester-Meredith & Marler, 2001; Bester-Meredith & Marler, 2003; Bester-Meredith, Young, & Marler, 1999; Fuxjager, Mast, Becker, & Marler, 2009; Marler, Bester-Meredith, & Trainor, 2003; Oyegbile & Marler, 2005) and highly territorial (Ribble, 1990; Ribble & Salvioni, 1990) California mouse (Peromyscus californicus) is an ideal system for examining competitive behavior. Males exhibit highly stereotyped contests that rarely result in injury (Eisenberg, 1962) and display a strong, natural winner effect (Oyegbile & Marler, 2005) compared with other Peromyscus species (Oyegbile & Marler, 2006). Winner effects in California mice have been demonstrated following three previous victories (Fuxjager et al., 2009; Fuxjager et al., 2011; Oyegbile & Marler, 2005), and contributing factors to the development of the winner effect such as T, fighting experience, and contest location, have been disentangled and characterized. Full winner effects are expressed only in males that have experienced previous victories (Fuxjager et al., 2011), a postvictory surge in T (Fuxjager et al., 2011; Marler, Oyegbile, Plavicki, & Trainor, 2005; Oyegbile & Marler, 2005; Trainor et al., 2004), and fight their contests in their home cages (Fuxjager et al., 2009). None of these components alone is sufficient to induce a full winner effect (Fuxjager et al., 2009; Fuxjager et al., 2011). Recently, male California mice have been shown to develop a conditioned place preference (CPP) to T (Zhao & Marler, 2014), consistent with findings in other species (Johnson & Wood, 2001; Alexander, Packard, & Hines, 1994; Packard, Cornell, & Alexander, 1997; Wood, Johnson, Chu, Schad, & Self, 2004). This suggests that a postvictory rise in T in this species may be rewarding similar to the...
way in which food, sex, and drugs are rewarding and also elicit CPPs (for review, see Tschenkte, 1998, 2007).

Based on the literature referenced previously, our goal in the current study was to examine how receptor blockade after a male–male contest influences future aggression in the aggressive California mouse. Specifically, we aimed to examine whether D1- and D2-like receptor blockade regulates the formation of the winner effect. We predicted that D1-like receptor blockade with SCH-23390 would decrease the probability of winning and offensive aggression in a final test contest. Because studies implicate D2-like receptor regulation of both offensive and defensive behavior, we expected that administration of the selective D2 antagonist raclopride would also reduce winning and attack behavior and increase expression of defensive behaviors.

Materials and Methods

Subjects

We used 109 sexually naïve adult male P. californicus mice ranging in age from six to 12 months, reared in our laboratory colonies at the University of Wisconsin-Madison. Of these, 32 were randomly selected experimental animals, 45 individuals were intruders during the training phase, and 32 were intruders during the final testing phase. Sixty-four female mice were used as mates for experimental males and final test intruders. Animals were maintained in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. Animal treatment and research protocols were approved by the University of Wisconsin-Madison College of Letters and Sciences Institutional Animal Care and Use Committee (IACUC; L0021-0-03-10). Prior to the experiment, animals were housed in same-sex cages (48.3 cm × 26.7 cm × 15.6 cm), with food and water available ad libitum. During the experiment, focal males were randomly selected, paired with a female, and housed in large Plexiglas observation cages (30 × 50 × 30 cm) that contained a nest box and tube for enrichment and access to food and water ad libitum. Animals were kept under a 14/10-hr light/dark cycle. We conducted behavioral tests under dim red light 30 min after the initiation of the dark phase.

General Procedures

Thirty-two (n = 8/condition) male P. californicus were randomly assigned to one of four conditions: D1-like antagonist, D2-like antagonist, vehicle, or handle. The contests in this experiment followed the resident-intruder paradigm described in Oyegbile & Marler, 2005. In the training phase (described later), each of the experimental males experienced an aggressive contest against a smaller, sexually inexperienced intruder, followed by a peripheral injection or a handling experience for a total of three training experiences. During the testing phase (see later), experimental males engaged in a single aggressive contest against an opponent with a slight competitive edge. All contests were 10 min unless otherwise terminated for injury (presence of a wound) by the experimenter. California mice display stereotyped aggressive behavior (Eisenberg, 1962), and accordingly, no contests resulted in physical injury.

Training Phase

On Day 1, focal males were weighed and paired with a female. On Day 11, each male and its mate were placed in a large Plexiglas observation cage (30 cm × 50 cm × 30 cm) with two compartments (30 cm × 29 cm × 30 cm and 22 cm × 29 cm × 30 cm) and two small holes to allow for passage between chambers. Prior to each of the training contests that occurred on Days 13, 15, and 17, experimental males were placed in an open field chamber (15 × 15 × 15 in.) with a 1-in square grid marked floor to measure locomotion as is routine in pharmacological studies. The number of lines crossed, as well as number of rears (standing on hind legs), were recorded and analyzed. Following the open field test, males were returned to their observation cages. Following a 5-min rest period, female mates were removed from the cage, and an opaque divider was inserted to separate the experimental animal (resident) from the intruder that was then placed into the cage. To ensure winning during the training phase (Days 13, 15, and 17), experimental males fought contests against younger, smaller (M = 3.98 g ± SE = 0.37) intruders in their home cage (e.g., home field advantage; Bester-Meredith et al., 1999; Fuxjager et al., 2010; Trainor & Marler, 2001). Furthermore, because sexual experience (Wang, Hulihan, & Insel, 1997) can increase aggressive behavior, intruders were sexually inexperienced. Each intruder was randomly assigned and used no more than twice. The intruder was allowed 2 min for acclimation to the cage. The 10-min contests began when the divider was removed. Contests were videotaped and later scored by an observer blind to the test conditions. We recorded the number of attacks (face-body contact), chases, WWE, wrestling bouts, jumps away from an opponent (Markham, Yang, Blanhard, & Blanchard, 2005), and freezes (remaining in a fixed position after a fight; Scholtens, Van Haaren, & Van De Poll, 1988), as well as latency to approach an intruder, to attack an intruder, and to freeze. No contests resulted in physical injury to the mice. To examine the effects of postvictory DA receptor blockade on future competitive behavior, focal males were injected with either a D1-like antagonist, D2-like antagonist, vehicle, or experienced handling (animals were removed from the cage in the same manner and for the same amount of time as animals that were injected) immediately following each training contest.

Testing Phase

To examine the effect of DA receptor blockade on competitive behavior and the winner effect (Day 19), the focal animals were exposed to an aggressive contest against an opponent with a slight competitive edge. These unfamiliar intruders were larger than the residents (M = 2.94 g ± SE = 0.37), sexually experienced, and had won a previous contest prior to the test contest. These parameters were selected to examine the extent of the winner effect as described in Oyegbile and Marler (2005). Each opponent in the final contest was used only once. The outcome of the final contest was determined by the mouse who initiated attacks that elicited submissive behavior from its opponent. No contests resulted in physical injury to the mice during the testing phase.

DA Antagonists

Males were randomly assigned to the following drug treatments: 0.5 mg/kg SCH-23390 (D1 antagonist), 0.3 mg/kg raclopride (D2...
antagonist), saline (vehicle), or handling identical to the injection-treated animals (handling). Doses were chosen from the lower end of doses used in previous aggression research in mice (Rodríguez-Arias, Pinazo, Miñarro, & Stinus, 1999), and because the doses differ between antagonists, comparisons between drug treatments were not made beyond omnibus testing. All drugs were administered via intraperitoneal injection immediately following an aggressive contest.

Behavioral Analysis

Videotaped aggressive behaviors were scored by an observer blind to the treatment groups. We examined the frequency of attacks, chases, and wrestling bouts. The observer also recorded latency to approach an opponent and latency to attack an opponent. In addition, we examined defensive behaviors in residents (experimental males) because one hypothesis suggests that DA may regulate aggressive behavior through the expression of defensive behavior (Sweidan et al., 1991).

Manipulation Checks

In the current study, we administered DA antagonists after, but well before, each successive contest to influence DA receptor activity without disrupting the required winning experiences. We measured locomotor activity in an open field test and aggressive behavior across the training contests to rule out side effects of the blockers.

Statistical Analysis

Data were analyzed using Kruskal-Wallis exact tests, followed by Mann-Whitney U tests for comparisons between groups. To assess data with greater variability, such as latency to engage in a particular behavior, we used median tests followed by Mann-Whitney U tests for comparisons between groups of interest. Tests of a priori hypotheses (comparing antagonist treated animals with controls) were conducted using Bonferroni adjusted alpha levels of 0.025 per test (0.05/2). Mixed-groups factorial analyses of variance (ANOVA) were used to assess behavior across the training sessions (manipulation checks). All statistical analyses were conducted using SPSS (version 19.0, SPSS, Inc., Chicago, IL).

Four animals were excluded from our analyses because of failure to win any contests during the training phase, resulting in a total sample size of 28 mice in our analyses (D1 = 6, D2 = 8, vehicle = 7, and handling = 7).

Results

Because no differences between vehicle and handled animals emerged in either contest outcome, \( \chi^2(2, N = 14) = 17.50, p = .46 \), or any of the above-mentioned behaviors (all \( p > .1 \)), we combined these data in further analyses (Cohen & Cohen, 1983). From this point forward, will refer to the combined group as the control.

We examined the effects of DA receptor blockade on a suite of competitive behaviors as well as the formation of the winner effect. As predicted, we found significant effects of treatment on the number of number of attacks, initiated chases, and test contest outcome (winner effect).

Manipulation Checks

Open field locomotor activity across all contests. As predicted, because antagonists were administered after an encounter, but 48 hr before subsequent aggression tests, we found no significant effect of treatment on either the number of lines crossed, \( F(2, 26) = 0.581, p < .56 \), or rears, \( F(2, 28) = 0.773, p < .47 \), in the open field tests.

Attack behavior across training contests. As a manipulation check, a mixed-groups factorial ANOVA was performed to examine the effects of treatment group and time (training experience) on the latency to attack and number of times the focal male attacked his opponent. No differences were found between groups in several measurements of aggression during the training contests with less competitive intruders. While all groups displayed a significant decrease in the latency to attack an opponent over the three training sessions, \( F(2, 21) = 5.331, p = .01 \), there was no difference in attack latency among groups, \( F(2, 21) = 0.021, p = .98 \). There was no reduction in number of attacks expressed by the groups over the training contests, \( F(2, 21) = 0.389, p = .68 \). Furthermore, there was no significant difference in number of attacks during training between the groups, \( F(2, 21) = 29.74, p = .3 \). The effect of treatment on attack behavior was not revealed until the final contest (see below) in which the intruder was more evenly matched with the focal animal, as described earlier.

Chasing behavior across training contests. A mixed-groups factorial ANOVA with post hoc contrast tests was performed to examine the effects of both treatment group and time (training experience) on the number of times the focal male chased his opponent. There was no main effect of treatment group, \( F(2, 21) = 1.051, p = .37 \), on chasing behavior. The number of observed chases was significantly different across training contests, \( F(2, 21) = 3.176, p = .05 \), and post hoc tests indicate a significant quadratic contrast, \( F(1, 21) = 5.973, p = .025 \). Chasing behavior was more frequent in training contest 2 \( (M = 5.43) \) compared with contest 1 \( (M = 3.62) \) and 3 \( (M = 3.05) \). Overall, there was no evidence of a decrease in aggressive behaviors.

Winner Effect in the Final Contest

A Kruskal-Wallis test was conducted to test for winner effects (repeated wins lead to an increased ability to win against a final intruder with a slight competitive edge) across treatment groups (control, D1, and D2). The test, which was corrected for tied ranks, was significant, \( \chi^2(2, N = 28) = 14.60, p < .001 \). Follow-up Mann-Whitney tests were conducted to evaluate pairwise differences between the control and other treatment groups, with Type I error controlled across tests by using Bonferroni corrections of the significance level \( (p = .05/2 = 0.025) \). The results of these tests indicated a significant difference between the controls and those treated with a D1 antagonist \( (z = -3.49, p < .001) \). There was a decreased ability to win the final contest in D1 antagonist-treated animals (mean rank = 4.5) compared with control animals (mean rank = 13.07). We also found a significant difference in winning ability between D2 antagonist -treated and control males \( (z = -2.78, p < .005; \text{Figure 1}) \). Animals treated with a D2-like antagonist (mean rank = 7.25) won fewer contests than control animals (mean rank = 13.93).
Social Investigation in the Final Contest

A median test was conducted to evaluate differences in latency to approach an opponent among the treatment groups (control, D1, and D2). We found no significant differences, \( \chi^2(2, N = 29) = 3.45, p = .18 \).

Offensive Behavior in the Final Contest

**Attack.** A Kruskal-Wallis test was conducted to evaluate differences among the treatment groups (control, D1, and D2) on the mean number of times the focal animal attacked his opponent (mouth–body contact). The test, which was corrected for tied ranks, was significant, \( \chi^2(2, N = 28) = 10.1, p = .003 \).

Follow-up Mann-Whitney tests were conducted to evaluate pairwise differences in attack behavior among the control and the other treatment groups, controlling for Type I error across tests using Bonferroni corrections (significance level of \( p = .05/2 = 0.025 \)). The results of these tests indicated a significant difference between the control animals and those treated with a D1 antagonist (\( z = -2.866, p = .003 \)), but only a nonsignificant trend for those treated with a D2 antagonist (\( z = -2.02, p = .04 \); Figure 2). Control animals attacked their opponents more (mean rank = 12.96) than either D1-like antagonist or D2-like antagonist treated animals with average ranks of 4.75 and 7.81, respectively.

A median test was conducted to evaluate differences among the treatment groups (control, D1, and D2) in the latency to attack an opponent. We found no significant differences between the groups, \( \chi^2(2, N = 28) = 2.31, p = .31 \).

We used a Kruskal-Wallis test to examine differences between the treatment groups (control, D1, and D2) in the number of wrestling bouts. We found no significant differences between the groups, \( \chi^2(2, N = 28) = 0.46, p = .80 \).

**Chase.** A Kruskal-Wallis test was conducted to evaluate differences among the treatment groups (control, D1, and D2) on the mean number of initiated chases. The test, which was corrected for tied ranks, was significant, \( \chi^2(2, N = 29) = 6.53, p = .03 \).

Follow-up Mann-Whitney tests were conducted to evaluate pairwise differences between vehicle and the other treatment groups, controlling for Type I error across tests by using Bonferroni adjusted alpha levels of 0.025 per test (0.05/2). The number of chases initiated by focal males was lower for the D1 group with an average rank of 5.83 compared with the controls with an average rank of 12.5. There was no difference between the control animals and those treated with a D1-like antagonist (\( z = -1.92, p = .12 \)).

Defensive Behaviors in the Final Contest

**Freezing behavior.** No differences among the treatment groups (control, D1, and D2) on the number of freezes or latency to freeze in response to an opponent were observed as no resident mice displayed freezing behavior in these tests.

**Jumping away.** A Kruskal-Wallis test was conducted to evaluate differences among the treatment groups (control, D1, and D2) on the number of times a focal animal jumped away from its opponent. The test revealed no significant differences, although there was a nonsignificant trend, \( \chi^2(2, N = 28) = 4.52, p = .09 \).

Discussion

Our experiment demonstrates an essential role of the dopaminergic system in the expression of the winner effect. Postcontest inactivation of DA receptors via peripheral administration during

![Figure 1](image1)

*Figure 1. The winner effect (percent that won a final contest) was influenced by D1-like and D2-like receptor blockade. Hatched bars represent control males, open bars represent SCH-23390 treated males and dark bars represent raclopride treated males. * Significant differences in behavior between control and D1 antagonist and D2 antagonist treated males; \( p = .001 \) and \( p = .008 \), respectively.

![Figure 2](image2)

*Figure 2. D1-like and D2-like receptor blockade influenced number of attacks (dark bars) and chases (white bars) delivered by focal males (Mean ± SE). * Significant differences in attack behavior and chasing behavior between control and D1 antagonist treated males; \( p = .001 \) and \( p = .01 \), respectively. Using Bonferroni adjusted alpha levels of 0.025 per test (0.05/2), the + indicates a nonsignificant trend in attack behavior between controls and D2 antagonist treated males; \( p = .04 \).
the training phase, while not interfering with training behavior with a less competitive opponent, directly influenced a male’s ability to mount a win against an evenly matched opponent in the test contest. No males treated with the D1-like antagonist were victorious in the final contest. In a similar manner, the percentage of males that won the test contest was significantly reduced by inactivation of D2-like receptors compared with control males. Our results provide mounting support for DA’s contribution to aggressive behavior and most significantly compliment recent work indicating a potential role for both D1- and D2-like receptors in the regulation of the winner effect (Schwartzer et al., 2013). These results suggest that the widely observed increases in DA following aggressive contests may function to promote future winning ability.

It is unclear how activation of DA receptors may enhance the development of the winner effect, although at least two hypotheses have emerged involving reinforcing effects as well as the possibility of learning. The first hypothesis is that the postcontest surge in T may be rewarding or reinforcing. Rodents exhibiting CPPs to T (Alexander, Packard, & Hines, 1994; Arnedo, Salvador, Martinez-Sanchis, & Gonzalez-Bono, 2000; Packard et al., 1997) will work to gain access to exogenous T and show diminished strength of the CPP following administration of a DA antagonist (Johnson & Wood, 2001; Wood, 2002; Wood et al., 2004). Male California mice can also exhibit CPPs to T depending on experience and environmental context (Zhao & Marler, 2014). Other researchers have suggested that aggression itself may be rewarding (Couppis & Kennedy, 2008; Couppis et al., 2008; Farrell & Wilczynski, 2004). We found that aggression and winning were diminished in the final test contest, which supports the possibility that T and/or aggression may be rewarding. Although we did not manipulate T in the current study, recent tests of the winner effect indicate that in the absence of T, the experience of winning alone is not sufficient to induce a full winner effect (Fuxjager et al., 2011). This leads us to speculate that T may mediate the association between aggression and DA.

The second hypothesis is that DA is involved with reward learning. According to the principles of associative learning, a previously neutral stimulus (aggressive behavior), when presented with a rewarding stimulus (T) will become reinforced (DA) and the expression of the behavior increased. An escalation of aggressive behavior is an observed hallmark of the winner effect (Fuxjager et al., 2011; Oyegbile & Marler, 2005). With blockade of DA receptors, (i.e., removal of the neurobiological reinforcer), aggressive behavior, according to learning principles, should be diminished. Although we did not observe a reduction in aggression during the training contests, aggression and winning was diminished in the final test contest. Our findings are consistent with associative learning theory. Although we found that both D1 and D2 receptor blockade reduces aggression and winning ability, a majority of studies implicate D1 and not D2 receptors in reward learning (for review see Beninger & Miller, 1998).

An alternative, though related interpretation of our results, is that DA may be critically important for the integration of information across successive training victories that is necessary for an individual to compete successfully against an evenly matched opponent. T has been shown to serve a similar function in humans (Wright, Edwards, Fleming, & Dolan, 2012). Participants that were given T between training sessions on a cognitive task learned more (as measured by performance) than placebo or control group members suggesting a role for T in “off-line” learning. Although aggressive behavior was reduced in the test contest, it is unclear whether that was a consequence of extinction or a failure to consolidate information vital to a win against an evenly matched opponent. If, as we suspect, DA is important for the integration of information accumulated during training wins, future studies should vary the number of training contests prior to a test contest to rule out the possibility of extinction.

Finally, our data suggest that the social context may be an important motivator for males to engage in aggressive behavior. We found that aggressive behavior was not altered by antagonist treatment during training, but declined sharply in the test contest. Although speculative, it is possible that males will continue to behave aggressively without reinforcement when the costs are relatively low (against a smaller opponent). Whereas males may be less motivated to engage with a more substantial opponent, such as the intruder in the test contest, when the associated costs are high (Dufty, 1989; Marler & Moore, 1989; Silverman & Dunbar, 1980). Recent studies aimed at understanding the exact contributions of the different DA receptor subtypes have implicated D1 receptors, as mediators of the motivational aspects of appetitive behavior (Young & Geyer, 2010). Chasing behavior is a plausible indicator of motivation because resident males in a monogamous species are expected to be highly motivated to exclude intruding males from their territories. Consistent with the D1 regulation of motivation hypothesis, we found that chasing behavior was diminished in the test contest by treatment with a D1-like receptor SCH-23390 but not by treatment with the D2-like antagonist, raclopride. These findings are also consistent with recent work suggesting D1-like receptor’s indirect effects on aggression through a reduction in social interest (Schwartzer & Melloni, 2010). We did not, however, observe any differences between the groups in attack latency during the test contest that may also reflect social interest.

Studies of D2-like receptors have emphasized a direct role in the regulation of aggression through changes in both offensive and defensive behavior. Although we did not observe an increase in the defensive behaviors measured, as was anticipated based on findings by Sweidan et al. (1991), our results provide evidence for direct regulation of aggression as both winning ability and attack behavior were decreased by treatment with raclopride during the training phase.

One caveat is that we delivered antagonists via a peripheral route of administration rather than targeting specific structures within the social brain network. Although further studies are needed to identify specific regions involved in the integration of information between contests, our results are consistent with recent studies examining the antiaggressive effects of DA antagonists administered before an aggressive contest via a central route of administration.

**Conclusions**

The findings of the current study provide further evidence for contributions of both D1-like and D2-like receptors in the regulation of aggressive behavior. Given our approach of disrupting DA receptors following aggressive contests, we show that both D1 and
D2-like receptor subtypes contribute to aggression and winning ability in a test contest in the absence of locomotor deficits. While we did not distinguish between hypotheses for how DA is facilitating development of the winner effect, it is unlikely that it is a simple increase in aggression in all contexts because this was not observed during training. In point of fact, we provide support for several hypotheses concerning DA's role in facilitating future winning behavior including theories of motivation, reward and social interest. Most important, our work leads us to posit that postcontest DA receptor activation may serve to integrate information necessary for future winning. This study significantly improves our understanding of the importance of DA in the development of the winner effect through winning experiences.

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