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Ventral Striatum Dopamine D2 Receptor Activity Inhibits Rat Pups' Vocalization Response to Loss of Maternal Contact

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Abstract

Most mammalian infants vocalize when isolated. The vocalization promotes caregiver proximity, which is critical to survival. If, before isolation, a rat pup has contact with its dam, its isolation vocalization rate is increased (maternal potentiation) relative to isolation preceded only by littermate contact. Prior work showed that systemic administration of a D2 receptor agonist blocks maternal potentiation at doses that do not alter baseline vocalization. In this study, infusion of quinpirole (2 µg/side) into the nucleus accumbens also blocks maternal potentiation. Infusion of the accumbens with the D2 antagonist raclopride (4 µg/side) prevents systemic quinpirole from blocking potentiation. Quinpirole infusion in the dorsal striatum did not affect maternal potentiation and infusion of raclopride in the dorsal striatum did not reverse the block of maternal potentiation by systemic quinpirole. Vocalization results after a second vehicle infusion on a given day are no different than the results following an initial vehicle infusion, so experimental design can not account for the effects of drug infusions. Because activity level was increased by both dorsal and ventral striatum infusions, activity level can not account for the results.

Keywords

infant vocalization; social behavior; dopamine; D2; ventral striatum

Mammalian infants' proximity maintaining behavior with caregivers is critical to their survival, and likely contributes the formation of social bonds (Bowlby, 1969; Francis, Diorio, Liu, & Meaney, 1999; Harlow, Dodsworth, & Harlow, 1965; Hofer, 1994). From birth until weaning, most infant mammals vocalize when socially isolated (Gurski, Davis, & Scott, 1980; Hennessy & Ritchey, 1987; Hofer, Brunelli, & Shair, 1993; Kalin, Shelton, & Snowden, 1992; Rifkin & Glickman, 2004). Isolation-induced ultrasonic vocalizations (USV) of rat pups guide retrieval by the dam (Brunelli, Shair, & Hofer, 1994) and elicit other maternal behaviors (Hashimoto, Saito, Furudate, & Takahashi, 2001; Jans & Leon, 1983).

Brief interaction with a potential caregiver just before social isolation increases rate and intensity of vocalization, an effect termed "potentiation" (Hofer, Brunelli, & Shair, 1994; Kraebel, Brasser, Campbell, Spear, & Spear, 2002; Myers et al., 2004). The importance of

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USV potentiation for investigation of mechanisms by which social bonds are formed lies in its social specificity and, in at least some forms, in its dependence on experience. For dam-reared pups, potentiated USV responses to isolation are elicited only by adult females; not by interactions with littermates, home cage bedding, or neutered adult males, even though all these stimuli reduce vocalization when they are placed with the isolated pup in the test chamber (Brunelli, Masmela, Shair, & Hofer, 1998; Hofer et al., 1994). Importantly, if pups have been reared with their sire, adult males can elicit USV potentiation (Brunelli et al., 1998; Shair, 2007).

Dopamine has been implicated in social behaviors. Adult pair bonding (Wang et al., 1999) and maternal behavior (Stern & Lonstein, 2001) both depend on dopaminergic activity. Regarding infant pup vocalization, agents that activate dopaminergic receptors decrease isolation calling in rat pups (Dastur, McGregor, & Brown, 1999; Kehoe & Boylan, 1992). Recently, we have shown that D2-family dopamine receptor activation has a more specific role on maternal potentiation. Quinpirole, a D2-family agonist, selectively blocks the potentiated vocalization rate at dosages that do not reduce calling by pups during an initial isolation (Muller, Brunelli, Moore, Myers, & Shair, 2005).

The brain areas that underlie the effects of quinpirole on maternal potentiation are unknown. One candidate, the nucleus accumbens, is the principal component of the ventral striatum along with the olfactory tubercle. The accumbens is known to be involved in other kinds of vocalization (Burgdorf, Knutson, Panksepp, & Ikemoto, 2001; Doupe, Perkel, Reiner, & Stern, 2005; Thompson, Leonard, & Brudzynski, 2006). Furthermore, the nucleus accumbens and dopaminergic activity therein are implicated in motivated behavior (Ikemoto & Panksepp, 1999; Salamone, Correa, Mingote, & Weber, 2005; Smith, 2004), including the formation of social bonds in adult voles (Aragona, Liu, Curtis, Stephan, & Wang, 2003). Therefore, in this study we targeted the accumbens to test whether D2 receptor activation there blocks maternal potentiation and D2 receptor inactivation there reverses the effect of systemically administered agonist.

Method

Subjects

Pregnant females from our Wistar breeding colony (founders obtained from Hilltop Farms, Scottsdale, PA) were brought to a satellite housing room 3 days before expected delivery date. The day of birth was considered postnatal Day (PND) 0. Pups were culled to five males and five females on PND 1. Two to four pups were selected at random to receive cannula surgery and one was assigned to a systemic-injection group. Pups were selected without regard to sex. In previous work, there was no significant effect of sex in 14 day old pups on vocalization rate nor on the maternal potentiation of the vocalization rate (Muller et al., 2005; Shair, Brunelli, & Hofer, 2005). All animal care and procedures were consistent with federal regulations and approved by the institutional animal care and use committee.

Experimental Design

After a litter was culled, the dam and litter remained together in the homecage, undisturbed except for routine animal facility care until PND 10 when cannula implantation surgery, described below, was performed. Animals recovered with littermates and dam on PND 11. Pups were tested on PND Days 12, 13, and 14. On the testing days, after removing the dam, the home cage was placed on a thermostatically regulated heating pad to maintain the home cage floor at 33° C. On the first day of testing, the dam was anesthetized with 0.12 ml/100 g body weight of a ketamine (80 mg/ml) and xylazine (4 mg/ml) solution and supplemented as necessary throughout the behavioral testing. On the last testing day (when recovery was not

required), the dam was anesthetized with 2 g/kg urethane. On each testing day, each pup was tested twice. On the first testing, the pup received a vehicle (physiological saline) infusion via the brain cannula (the procedure is described below). The pup was replaced in the litter pile for 10 to 30 min. Then, the pup was tested in the maternal potentiation behavior protocol (described below). After completion of the first test, the pup was returned to its litter pile for 30 min. Then, a second solution appropriate to the assigned group was infused. As with the vehicle infusion, the pup was returned to the litter pile and tested later. A pup was tested with different infusion-condition assignments on each testing day. Experimental infusion conditions were counterbalanced across days for different pups. At the end of testing each day, the dam was returned to the homecage when fully ambulatory and alert, and the homecage was returned to the housing facility. After the end of the third day of testing, the litter was euthanized by exposure to CO₂. The brains of cannulated pups were recovered and processed as described below.

The following experimental conditions were included: systemic (i.p.) quinpirole only, quinpirole infused in the nucleus accumbens, quinpirole infused in the dorsal striatum, raclopride infused in the nucleus accumbens plus systemic quinpirole, and finally, raclopride infused in the dorsal striatum plus systemic quinpirole. For each infused drug condition, there was a previous testing in the same pup with vehicle infusion into the same target. Table 1 indicates how drug conditions for an individual pup were arranged across days. Different combinations of drug infusions and days of testing were used depending on the cohort. In one additional control group, seven pups were given vehicle infusions into the nucleus accumbens on both the first test and the second test of the day.

Surgery

On PND 10, pups designated for surgery were anesthetized with isoflurane (Baxter, Deerfield, IL). When the pup was unresponsive to tail pinch, the skull was exposed and the animal was placed in a stereotaxic frame. Supplements of anesthetic were administered as needed throughout the surgery. Body temperature was maintained with a heating pad. Small holes were drilled in the skull above the nucleus accumbens. Guide cannulae (fashioned from 23 gauge hypodermic needles) were implanted bilaterally targeted at the nucleus accumbens. The target coordinates relative to Bregma were 0.9 mm anterior, 3.65 laterally, and lowered –2.0 mm from the skull surface at a 15 degree angle from vertical such that the bottom of the cannula was closer to the midline than the hole in the skull through which it entered. The coordinates were designed to avoid passage through the lateral ventricles and based on previous experience with pups of this age. A mixture of powdered cement (Cranioplastic Powder, Plastics One) and its solvent (Ortho Jet Liquid, Lang Dental Manufacturing, Wheeling, IL) was applied around the cannula and made contact with surrounding undamaged skin to seal the wound. After the surgery, topical antibiotic was applied. The pups recovered in a cage on top of a heating pad maintained at 35.5° C. The guide cannulae were painted with an unpleasant tasting nail polish (“Thum” Oakhurst Company; Levittown, NY) to discourage biting by the mother. The rats were then returned to their homecage and dam. Surgeries were begun at noon, the rats recovered the next day, and testing began at noon of the second day after surgery (PND 12). Weights were taken on the day of surgery and again at testing to confirm that all rats recovered sufficiently to gain weight in the recovery period.

Drug Preparation

Concentrated stock solutions were prepared by dissolving quinpirole and raclopride in deionized water. Physiologic saline (0.9% NaCl) was used to mix with stock drug solutions to prepare the final concentrations. Aliquots of quinpirole at 5 mg/ml and raclopride at 10 mg/ml were prepared for local infusion into the nucleus accumbens and stored at –70° C.

Quinpirole at 1 mg/kg concentration for systemic injections was drawn into 1 ml syringes with 30-g needles and frozen at -70°C . The injected quinpirole syringes were prepared for a delivery volume of 0.1 ml per 30 g of body weight. Frozen drug preparations were removed from the freezer and thawed for at least 20 min before experimental use. All drugs were shielded from light exposure.

Drug Infusion and Injection

Systemic-quinpirole-only condition: a pup that had not been implanted with cannula was picked up and injected i.p. with systemic quinpirole 1 mg/kg. The pup was returned to its homecage and then tested 30 min later.

Micro-infusion conditions: infusion cannulae were fashioned from metal of 30 gauge gaud 1 inch hypodermic needles. They were cut so that when fully inserted, the infusion cannula for the nucleus accumbens extended 5 mm beyond the tip of the guide cannula and the dorsal striatum 1.5 mm. The pup to be infused was picked up from the litter pile, carried to a separate room, and held in the palm of one hand during the infusion procedure. Both guide cannulae were cleared by inserting a 30 gauge needle 3/4 of the way down each cannula in its turn. Then, on one side, the infusion cannula was inserted. The cannula was connected by Polyethylene tubing to a 10 μl syringe (Hamilton, Reno, NV). The liquid volume, 0.4 μl , was infused at a steady rate over 100 s using a syringe pump (model sp100i; World Precision Instruments, Sarasota, FL). The infusion cannula was left in place an additional 1 min to allow diffusion of the contents away from the cannula tip. The procedure was repeated for the other side. After infusion, the pup was returned to its home cage and litter pile. The cannula was tested for blockage by running the pump again. If no liquid was noted at the cannula tip, it was presumed the drug was not adequately administered and the pup's data were excluded. In each infusion, the amount of drug in the 0.4 μl of saline was as follows: quinpirole 2 μg or raclopride 4 μg . Quinpirole and raclopride infusion concentrations were based on prior studies of these ligands infused into the nucleus accumbens (David, Sissaoui, & Abraïni, 2004; Ikemoto, Glazier, Murphy, & McBride, 1997; Samson & Chappell, 2003; Sederholm, Johnson, Brodin, & Sodersten, 2002). Animals infused with raclopride were given an i.p. injection of 1 mg/kg quinpirole 10 min later and tested 15 to 20 min after injection. Animals infused with quinpirole were tested 10 to 30 min after infusion.

Behavioral Testing

In each test, a pup was observed during two isolation periods of 2 min each with an intervening 2 min reunion with its anesthetized dam. This test for maternal potentiation has been used in many studies from our laboratory (Brunelli et al., 1998; Hofer et al., 1994; Hofer & Shair, 1978; Shair, Brunelli, Masmela, Boone, & Hofer, 2003). At the appropriate time after drug administration, each pup was picked up and placed in a clean polycarbonate box (18 cm long $\times$ 21 cm wide $\times$ 20 cm deep) and observed for 2 min (ISO1). The underside of the floor of the cage was marked to produce six equal squares. Immediately after ISO1, the pup was picked up, the anesthetized dam was put in the box, and the pup was placed back in the box in contact with the dam. After the 2 min reunion, the pup was picked up, the dam removed, and the pup re-isolated. Behavior was also recorded during the second isolation (ISO2).

Immediately after ISO2, those animals infused with quinpirole were tested in a cold environment. The pup was placed in an identical test box for 2 min and observed as before, except that this test box was in a 10°C chamber.

Behavioral observations: ultrasonic vocalizations were detected with a bat detector (Pettersson Elektronik, Uppsala, Sweden), transduced into an audible range and counted

with a silent, hand-operated counter by an observer with headphones. The number of squares crossed (a measure of locomotion), 360 degree turns in square, and rearing (raising of the head above shoulder level and one or both front paws raised off the floor against the wall or in the air) were summed to produce a measure of locomotor activity. At the conclusion of each pup's test, its axial temperature was measured.

Cannula Placement

Infused pups' brains were recovered after the experiment, fixed, sliced, mounted on slides, Nissl stained, and cover slipped to reconstruct cannula locations. For the experimental conditions in which the nucleus accumbens was targeted, data were included only if at least one cannula tip was in the core of the nucleus accumbens and the other was in the core, in the shell, or abutting one of those two structures.

Data Analysis

These experiments were designed to test specific a-priori hypotheses about whether (1) infusions of the D2 agonist quinpirole into the nucleus accumbens would block maternal potentiation of USV and whether (2) the effect of systemic quinpirole on maternal potentiation would be reversed by infusion of the D2 antagonist raclopride into the nucleus accumbens. As such, the critical statistical analysis was a comparison within each experimental group of the rates of vocalization of ISO1 and ISO2. To control for multiple comparisons, first an overall ANOVA was done for each of the quinpirole and raclopride and systemic quinpirole data sets, then the individual tests for potentiation are presented. Other tests are described when presented in the results.

The issue that differences between litters could confound interpretation of the results was addressed in the following manner. Most importantly for each of the two main hypotheses, comparison of vehicle and drug data were within subjects. That is, we compared the effect of drug to the effect of vehicle within the same animal. Second, in any given test there were multiple litters. Finally, to check for the remaining possibility of litter effects, by meaning the data of all pups in a litter, we performed an example ANOVA using litter as the unit of analysis to demonstrate that the conclusion did not change (see the results concerning the ANOVA of quinpirole infusions). After that, we used subject as the unit of analysis for the rest of the results (see Table 2 for the number of litters in each experimental condition).

Results

There were 56 pups from 19 litters included in the data analysis. Figure 1a shows a micrograph of an example of bilateral cannula placements in the accumbens. The cannula tip locations of the 31 animals that had acceptable placements targeting the nucleus accumbens can be seen in Figure 1b. Placements were all within the posterior half of the nucleus accumbens, and were mapped onto adult diagrams using the medial-lateral position of the anterior limb of the anterior commissure.

Quinpirole Infused Into the Ventral Striatum Disrupts Maternal Potentiation

An ANOVA of isolation-induced vocalizations by (1) isolation phase (ISO1 vs. ISO2), (2) saline versus quinpirole infusion, and (3) nucleus accumbens infusions versus dorsal striatum infusions was performed, in which isolation phase and drug presence were repeated observations and infusion locations were independent observations. There were 19 subjects from 10 litters received infusions into the nucleus accumbens; 15 subjects from 7 litters received infusions into the dorsal striatum. The critical test of interest was the three way interaction of isolation phase by drug presence by infusion location, which was significant $F(1, 32) = 9.4, p = .0044$. When the analysis was conducted using only mean values for each

litter (to control for litter effects), the three way interaction was also significant: $F(1, 15) = 5.12, p = .039$. Quinpirole infused into the nucleus accumbens blocked potentiation (paired $t(18) = 0.55, p = .59$), but in all other conditions potentiation was present (saline in nAc paired- $t(18) = 4.92, p < .001$; saline in dorsal striatum paired- $t(14) = 7.79, p < .001$; quinpirole in dorsal striatum paired- $t(14) = 3.66, p = .003$, see Figure 2). Mean vocalizations by isolation phase, from which the potentiation scores are derived, are presented in Table 2.

It is unlikely that the absence of potentiation in the nucleus accumbens-quinpirole infused condition can be explained by quinpirole's enhancement of vocalization in the initial isolation: quinpirole infusions (regardless of location) did significantly increase vocalizations in the initial isolation compared to their vehicle infusion levels, paired- $t(33) = 3.6, p = .001$. This result is reflected in the overall three-way ANOVA results regarding the trend toward a main effect of drug presence, and the significant interaction of drug presence with isolation phase. However, other results that demonstrate that the vocalization rate could increase above the enhanced initial isolation rate. When quinpirole was infused into the dorsal striatum, there was an increase in ISO1 vocalizations, but vocalizations went up significantly *further* in ISO2 (see Figure 2). Second, the quinpirole infused into nucleus accumbens subjects were able to increase the number of vocalizations significantly when tested in isolation in a cold environment, paired- $t(18) = 6.38, p < .001$ (see Figure 3). Further when a subset of vehicle data with ISO1 vocalizations comparably high to the quinpirole pups ($\#USV > 60$) was analyzed, there remained a significant increase in vocalizations in ISO2 above ISO1, $t(8) = 5.04, p = .010$.

Raclopride Infused in the Ventral Striatum Reverses Systemic Quinpirole's Block of Maternal Potentiation

That activation of D2 receptors administered either systemically or specifically targeted to the nucleus accumbens blocks maternal potentiation suggests activity of D2 receptors in this region is necessary to disrupt potentiation. In support of this hypothesis, local infusion of the D2 antagonist raclopride into the nucleus accumbens (10 $\mu\text{g}/\text{side}$) prevented systemic quinpirole's effect (see Figure 4). That is, blocking the activation of D2 receptors in the ventral striatum restored potentiation to levels comparable to saline treated controls, but blocking D2 activation in the dorsal striatum did not.

An ANOVA of isolation-induced vocalizations by (1) isolation phase (ISO1 vs. ISO2), (2) drug presence (saline vs. systemic quinpirole and local raclopride infusion), and (3) infusion location (nucleus accumbens vs. dorsal striatum) was performed, in which isolation phase and drug presence were repeated observations and infusion location was an independent observation. There were 10 subjects from 6 litters received infusions into the nucleus accumbens; 19 subjects from 8 litters received infusions into the dorsal striatum. The critical test of interest was the three-way interaction of isolation phase by drug presence by infusion location, which was significant, $F(1, 27) = 7.6, p = .010$. Quinpirole's disruption of potentiation was blocked by raclopride infusion in the nucleus accumbens. In other words, there was potentiation, paired- $t(9) = 3.56, p = .006$ (see Figure 4). Quinpirole's disruption of potentiation was unaffected by raclopride infusion in the dorsal striatum. In other words, there was no potentiation, paired- $t(18) = 1.34, p = .20$ (see Figure 4). There was potentiation in vehicle infused conditions: nucleus accumbens paired- $t(9) = 5.69, p < .001$; dorsal striatum paired- $t(18) = 6.65, p < .001$. Mean vocalizations by isolation phase, from which the potentiation scores are derived, are presented in Table 2.

There was a trend for vocalization in the initial isolation (ISO1) to be higher in the drug than vehicle condition, $F(1, 27) = 3.70, p = .07$. As previously discussed, higher initial vocalization rates are not a bar to further increases in vocalization. Indeed vocalizations did

go significantly higher in the critical test of interest. That is, when raclopride was infused in the nucleus accumbens before quinpirole was injected systemically, there was potentiation.

Repeated Testing Does Not Account for the D2 Ligand Effects on Potentiation of Isolation Vocalizations

The experimental design assumes that differences between the first and second test sessions on a given day are a result of vehicle versus drug infusions respectively. However, if there were effects of retesting on vocalizations because of factors like habituation, sensitization, or maturation, this would confound such interpretation. To address this issue, the experiment included one group in which seven pups from five litters were tested with vehicle infusions before both tests of the day. No effect of retesting on vocalizations was observed. There were no significant differences between initial-isolation vocalization rates (paired- $t(6) = 1.60$, $p = .16$) nor maternal potentiation (paired- $t(6) = 0.45$, $p = .67$) between the two tests (see Figure 5).

Motor Effects of D2 Receptor Stimulation Do Not Account for Potentiation Results

As in prior reports in which systemic quinpirole elevated behavioral activity level compared to vehicle infusion levels (Dastur et al., 1999), activity as measured by the combined activity score was increased by local infusion of quinpirole in either the nucleus accumbens or the dorsal striatum, paired- $t(18) = 5.20$, $p < .001$ and paired- $t(14) = 5.02$, $p < .001$, respectively (see Figure 6). Elevated activity level could not be the cause of disrupted potentiation because pups infused in either location showed increased activity, but only pups infused in the nucleus accumbens did not show potentiation of isolation vocalizations.

Thermal Effects of D2 Receptor Stimulation Do Not Account for D2 Inhibition of USV Potentiation

Axial temperature was lowered by both systemic and local infusion of quinpirole. An ANOVA of axial temperature by experimental group was significant $F(5, 67) = 10.81$, $p < .001$: temperatures (Celsius) after systemic quinpirole ($M = 33.0$), quinpirole infused in either nucleus accumbens (33.3) or dorsal striatum (33.4), as well as systemic quinpirole and raclopride infused in dorsal striatum (33.7) were all significantly lower than vehicle (36.1) (all p s $< .001$, Dunnett's post hoc comparisons against the vehicle retest group). The mean temperature of the systemic quinpirole and raclopride infused in the nucleus accumbens (34.8) was not significantly lower than vehicle. Although reductions in temperature can increase USV rate (Oswalt & Meier, 1975), as is also shown in the present study by the increased vocalization in the cold test, small changes of the magnitude similar to those produced by quinpirole here have not been found to affect vocalizations (Brunelli, Vinocur, Soo-Hoo, & Hofer, 1997; Shair, Masmela, Brunelli, & Hofer, 1997). Furthermore, in the current data, the reduction in potentiated rate of calling after quinpirole is opposite that predicted by an effect of reduced body temperature.

Other Results

Although already presented, here it is emphasized that implantation of brain cannula and infusion of vehicle did not disrupt maternal potentiation of isolation vocalizations. A combination of all first test data that followed saline infusion, regardless of site of infusion or day of testing, demonstrated that cannulated animals exhibited USV potentiation similar to uncannulated animals in prior work ($n = 70$, see Figure 7A; Muller et al., 2005). That is, there was a significant increase in vocalization in the re-isolation period (ISO2) as compared to the initial isolation (ISO1), paired- $t(69) = 10.90$, $p < .001$.

To test the hypothesis that raclopride infused in the nucleus accumbens would reverse the effects of systemic quinpirole, as an added control, a quinpirole-injection-only condition was included for comparison. Replicating the previous report (Muller et al., 2005), systemic administration of the D2 agonist quinpirole disrupted maternal potentiation. That is, there was no significant difference in vocalization rates between ISO1 and ISO2, paired- $t(18) = 0.36$, $p = .73$ (see Figure 7B). For the vehicle infusions and systemic quinpirole results, mean vocalizations by isolation phase, from which the potentiation scores are derived, are presented in Table 2.

Discussion

These experiments demonstrate a specific role for ventral striatum D2-family receptors in maternal potentiation of isolation-induced vocalization, a rodent affective behavior that is dependent on recognition of potential caregivers. The potentiation effect, in which a pup's vocalization rate is higher in an isolation preceded by brief maternal contact than in an isolation preceded by contact with other social stimuli, for example, littermates or home-cage shavings, is absent when D2 receptors are activated systemically (Muller et al., 2005). The present results show that activation of ventral striatum D2 receptors alone is necessary and sufficient to block the potentiation of isolation vocalizations. Infusion of quinpirole into the nucleus accumbens, but not the dorsal striatum, prevented potentiation. The systemic effect of quinpirole was blocked by inactivating D2 receptors with raclopride infused in the nucleus accumbens, but not the dorsal striatum. Thus, *inactivity* of D2 receptors in the ventral striatum is necessary for maternal potentiation.

The experiments included a number of observations that made it possible to eliminate some nonspecific explanations of the drug effects on maternal potentiation. Effects because of vocalization ceiling, body temperature, and activity level were ruled out.

We have interpreted the local infusion of raclopride as demonstrating that local inactivation of D2 receptors in the ventral striatum reverses the effects of systemic quinpirole on potentiation. We know from prior work that systemic raclopride does not disrupt potentiation and that systemic raclopride reverses the effect of systemic quinpirole (Muller et al., 2005). Thus, we had a specific hypothesis that raclopride locally would reverse the systemic quinpirole effects. We did not test the effects of raclopride infusions alone, without systemic quinpirole injections. Thus, it remains a possibility that raclopride in the ventral striatum might have an unanticipated effect, which could complicate the interpretation of the results from the raclopride infusions with systemic quinpirole injections.

The infusion target, the border between the accumbens core and lateral shell, is near other structures that may have also been exposed to the infused ligands, including other parts of the striatopallidal system (Heimer, Zahm, & Alheid, 1995) including the olfactory tubercle and the intermixed anterior portions of the ventral pallidum and the most ventral portion of the dorsal striatum, as well as neighboring structures including the endopiriform nucleus and the piriform cortex. Thus, although we can confidently exclude the dorsal striatum, the limits of the infusion parameters used here do not rule out the possibility that structures not part of the ventral striatopallidal system could have played a role in the results. Experiments using a smaller infusion volume coupled with radioactively tagged D2 ligands might be able to narrow the critical region.

Together, these results are convincing evidence of the importance of the ventral striatum in this infant vocalization behavior. Because the results imply that the pup's experience of dam contact and removal leads to a period of D2 receptor inactivity, another important future

direction is to measure dopamine levels in the region throughout the isolation, dam contact, and re-isolation phases of this experimental design.

The present results, as well as unpublished results showing that inactivation of the ventral striatum abolishes isolation-induced vocalization (Muller, Moore, Myers, & Shair), are consistent with the hypothesis that the nucleus accumbens is involved in mammalian *infant* vocalization. Much is known about the neuroanatomical structures involved in vocalizations of adult mammals and birds (Davis, Zhang, & Bandler, 1996; Doupe et al., 2005; Jurgens, 2002). The neuroanatomy of infant rat vocalization is likely consistent with that of adult vocalizations, including the cingulate, the basal ganglia, periaqueductal grey, and brainstem motor and sensory nuclei. Given the known role of the periaqueductal gray in both adult (Davis et al., 1996) and infant (Wiedenmayer, Goodwin, & Barr, 2000) vocalization, D2 receptors on striatal neurons with direct projections from the nucleus accumbens (shell) to the periaqueductal gray or indirect projections by way of the lateral hypothalamus may be critical (Heimer et al., 1995). D2-family receptors on striatal interneurons or on projection neurons that participate in the cortico-striatal-thalamic loops could also be important (Kelley, Baldo, Pratt, & Will, 2005). D2 receptor effects on fibers of striatal afferents may also play a role. In the adult striatum, the role of the D2 receptor in limiting glutamatergic inputs to projection neurons has been well-established (Bamford et al., 2004; for a review see Moore, West, & Grace, 1999). A set of glutamatergic inputs to the striatum could be specifically activated by an isolation subsequent to a brief maternal reunion, and glutamate release from these inputs could be suppressed by D2 receptor activation.

In summary, vocalization is virtually a universal mammalian infant response to social isolation. The modulation of isolation-induced vocalization by recent brief contact with potential caregivers is a relevant paradigm for understanding early-life social bonds. The neural basis of this behavior has been little studied. We recently found that a dopamine D2 agonist disrupts maternal potentiation. The current results show that dopamine D2-family receptor inactivity in the ventral striatum is required for the potentiation of vocalization in an isolation that follows brief maternal contact.

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Figure 1.

Cannula placement locations. (a) Micrograph of a coronal section through the nucleus accumbens in which angled cannula tracks and tips in the nucleus accumbens can be seen bilaterally. The tracks are faint and can be distinguished as lines, slightly darker than the surrounding tissue, running through the striatum, angling slightly medially as they descend; a rounder bulge of similarly darker color showing the terminal point of each cannula can be discerned in each hemisphere slightly medial and ventral to the anterior limb of the tract of the anterior commissure. (b) Adult rat atlas graphics adapted from Paxinos and Watson files (Paxinos & Watson, 2005). Figures from adult coordinates 1.2, 1.0, 0.7, and 0.48 mm anterior to Bregma are used as the most appropriate corresponding maps of the two week old rat pup cannula placements. Cannula tips in the ventral striatum are indicated by small gray ovals. The medial-lateral and dorsal-ventral extent of the dorsal striatum where infusions were made is indicated on the left side of the most anterior section by a large gray oval; such infusion locations were present bilaterally and throughout the indicated anterior-posterior range. Abbreviations: aca, anterior commissure, anterior limb; AcbC, accumbens core; AcbSh, accumbens shell; CPu, caudoputamen; DEn, dorsal endopiriform nucleus; ec, external capsule; gcc, genu of the corpus callosum; LAcSh, lateral accumbens shell; LV, lateral ventricle; SIB, substantia innominata, basal; VP, ventral pallidum. Adapted from *The*

Rat Brain in Stereotaxic Coordinates (4th ed.), G. Paxinos and C. Watson, 1998. Copyright 1998, with permission from Elsevier.

Figure 2.

Quinpirole in the ventral striatum blocks potentiation. Isolation-induced vocalizations are counted in two isolations, one preceded by contact with littermates (ISO1) and a second preceded by contact with the dam (ISO2). Potentiation scores are displayed on the y-axis, derived by subtracting ISO1 from ISO2. Unlike vehicle infusions and quinpirole infusions in the dorsal striatum, quinpirole infusions in the ventral striatum abolished potentiation. The interaction of change score by drug presence by infusion location was significant $F(1, 32) = 9.4$, $p = .0044$: quinpirole infused into the ventral striatum blocked potentiation (paired $t(18) = 0.55$, $p = .59$); but in all other conditions potentiation was present (saline in the ventral striatum paired- $t(18) = 4.92$, $p < .001$; saline in dorsal striatum paired- $t(14) = 7.79$, $p < .001$; quinpirole in dorsal striatum paired- $t(14) = 3.66$, $p = .003$). ns, not significant; *** $p < .001$. There were 19 pups in the ventral striatum group, 15 pups in the dorsal striatum group.

Figure 3.

Quinpirole disrupts vocalization increase in ISO2 but not in COLD condition. Number of vocalizations in a 2 min isolation phase are displayed on the y-axis. In animals that had received infusions of quinpirole, the number of isolation-induced vocalizations following dam-contact (ISO2), are compared to the number of vocalizations when re-isolated in a 10° C cold environment. There was a significant increase in vocalization in the cold, for both the group with infusions in the ventral striatum, paired- $t(18) = 6.4$, $***p < .001$, and in the dorsal striatum group, $t(14) = 3.6$, $p = .0027$ ($**p < .01$). The increase in cold induced vocalization in the ventral striatum group was present even though the quinpirole infusions

there had disrupted the increase in the number of vocalizations from the initial isolation to ISO2.

Figure 4.

Raclopride in the ventral striatum prevents the effect of systemic quinpirole on potentiation. See caption of Figure 2 for an explanation of the paradigm. Potentiation scores are displayed on the y-axis, derived by subtracting ISO1 from ISO2. In the vehicle infused groups, there was significant potentiation: ventral striatum paired- $t(9) = 5.69$, $p < .001$; dorsal striatum paired- $t(18) = 6.65$, $p < .001$. Raclopride infused in the dorsal striatum had no effect on systemic quinpirole's disruption of potentiation: ISO2 was not significantly different than ISO1, paired- $t(18) = 1.34$, $p = .20$. Raclopride infused in the ventral striatum prevented the systemic quinpirole effect; there was potentiation in the group, paired- $t(9) = 3.56$, $p = .006$. Thus, the absence of potentiation in the raclopride infused into the dorsal striatum and systemic quinpirole was significantly different than the potentiation seen in all other groups $F(1, 27) = 7.6$, $p = .010$. Data from ventral striatum infusions is from 10 subjects. Data from dorsal striatum infusions is from 19 subjects. ns, not significant; ** $p < .01$; *** $p < .001$.

Nucleus Accumbens Saline Infusions

Figure 5.

Retesting within a day does not alter isolation induced vocalization patterns. Mean vocalizations (with standard error bars) by infusion condition and infusion location. Each tick on the y-axes represents 50 vocalizations. See Figure 2 for an explanation of the paradigm. First Test and Retest vocalization data are shown for the group infused with vehicle into the ventral striatum before *both* tests ($n = 7$). Neither the amount of vocalization in the initial isolation nor the change in rate between ISO1 and ISO2 (potentiation) are significantly different between the two testings.

Figure 6.

Quinpirole infusions in both dorsal striatum and ventral striatum elevate behavioral activity. The y-axis represents the total count of locomotor activity behaviors (line crosses, rears, and turns in square summed across all three 2 min phases of testing). Quinpirole infused into either the ventral striatum (noted as nucleus accumbens) or the dorsal striatum elicited more locomotor activity than seen in their respective vehicle infusion conditions. *** $p < .001$. It can not be that increased locomotor activity caused decreased potentiation since activity was elevated by both infusion locations, but potentiation was only disrupted by infusion in the accumbens.

Figure 7.

Vocalization in control groups. See caption of Figure 2 for an explanation of the paradigm. (A) All Vehicle - Pooled data ($n = 70$) from all cohorts in which pups received vehicle (physiological saline) infusions in the initial testing of the day. Pups showed significantly increased vocalization (maternal potentiation) above the initial-isolation rate (ISO1) in the re-isolation following dam contact (ISO2), *** $p < .001$. Mean potentiation score is displayed with bar to indicate standard error. (B) Systemic Quinpirole ($n = 19$) blocks maternal potentiation. The D2 agonist administered systemically (1 mg/kg) prevented any increase in vocalization from ISO1 to ISO2, ns, change not significant.

Dopamine Ligand Conditions by Cohort

Table 1

Cohort	Day 1		Day 2		Day 3		Order of drugs	n
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2		
1	Vehicle	Raclopride in nucleus accumbens and systemic quinpirole						6
2	Vehicle	Quinpirole in nucleus accumbens	Vehicle	Raclopride in nucleus accumbens and systemic quinpirole			Counterbalanced	4
3	Vehicle	Quinpirole in dorsal striatum	Vehicle	Raclopride in dorsal striatum and systemic quinpirole			Counterbalanced	3
4	Vehicle	Quinpirole in nucleus accumbens	Vehicle	Quinpirole in dorsal striatum	Vehicle	Raclopride in dorsal striatum and systemic quinpirole	Counterbalanced	17
5	Vehicle	Vehicle					Counterbalanced across Days 1–3	7
All cohorts	One subject from each litter in all cohorts received a single interperitoneal injection of quinpirole (1 mg/kg) before behavioral testing on Day 1. No other testing was conducted on these subjects.							19

Note. Data presented in the paper arose from five cohorts that were subject to different testing conditions as shown here. On a given testing day, all animals were infused and tested twice. The first infusion of the day was always vehicle, the second infusion condition varied by cohort. For cohorts 2 through 4, in which animals were tested on more than 1 day, for the second infusion of the day, the sequence of drug conditions across days was counterbalanced, as indicated in column eight. In Cohort 5, the day of testing (corresponding to the other cohorts' Days 1, 2, and 3) was counterbalanced. The final column indicates the number of animals in each cohort.

Table 2
Vocalizations by Infusion Location, Drug Presence, and Isolation Phase for Quinpirole and Raclopride and Systemic Quinpirole

A						
Control conditions	Phase	Mean	SE	Subjects	Litters	
Vehicle infusions	ISO1	44.2	5.8			
	ISO2	94.5	7.2	70	19	
Systemic quinpirole	ISO1	68.5	17.0			
	ISO2	63.5	13.4	19	19	
B						
	Quinpirole	Mean	SE err	Subjects	Litters	
Nucleus accumbens	Vehicle	ISO1	43.7	13.3	19	10
		ISO2	101.5	17.5		
	Quinpirole	ISO1	83.3	17.6		
		ISO2	72.6	14.3		
Dorsal striatum	Vehicle	ISO1	42	11.1	15	7
		ISO2	72.9	12.5		
	Quinpirole	ISO1	74.4	17.8		
		ISO2	109.9	26.6		
C						
Raclopride and systemic quinpirole		Mean	SE	Subjects	Litters	
Nucleus accumbens	Vehicle	ISO1	34.8	9.2	10	6
		ISO2	106.4	11.0		
	Rac1 and Sys Quin	ISO1	46.8	12.2		
		ISO2	108.5	16.5		
Dorsal striatum	Vehicle	ISO1	59.9	12.5	19	8
		ISO2	105.7	14.7		
	Rac1 and Sys Quin	ISO1	80.3	17.3		
		ISO2	70.6	16.1		

Note. Vocalizations by isolation phase. Mean and *SEM* for the number of vocalizations during 2 min isolations are displayed for the initial isolation (ISO1) and re-isolation following dam contact (ISO2) for all testing conditions. Number of subjects is indicated in the column labeled "Subjects." Number of litters from which the subjects are derived is indicated in the column labeled "Litters." Three sets of results are distinguished. (A) Two control conditions: vehicle infusions - all vehicle infusion data combined from all data sets are shown here; systemic quinpirole - animals that received only systemic

injections (and were not implanted with cannula). (B) Eight infused Quinpirole conditions; data are compartmentalized by infusion location (nucleus accumbens or dorsal striatum) and drug status (vehicle or quinpirole). (C) Eight infused Raclopride and Systemic Quinpirole conditions; data are compartmentalized by infusion location (as above) and drug status (vehicle or infused raclopride followed by systemic quinpirole).