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Behavioral differences between selectively bred rats: D_1 versus D_2 receptors in yawning and grooming

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Abstract

We used SKF 38393 and quinpirole for determining whether activation of D_1 and D_2 receptors, respectively, is involved in behaviors of rats selectively bred for high or low rates of yawning. After injection of SKF 38393, yawning diminished more markedly in high-yawning (HY) than in low-yawning (LY) rats, whereas this drug increased the number and duration of grooming episodes similarly in both strains. After injection of quinpirole, yawning increased more markedly in HY than in LY rats, whereas this drug decreased the number and duration of grooming episodes similarly in both rat strains. After coadministration of SKF 38393 and quinpirole, yawning increased similarly in both rat strains, whereas the combination of drugs failed to reliably affect grooming behavior. We interpret our findings as indicating that D_2 receptors are more important than D_1 receptors for differences in yawning behavior between HY and LY rats.
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Keywords: Selectively bred rats; Grooming; Yawning; Dopamine; SKF 38393; Quinpirole

1. Introduction

Yawning is an infrequently occurring behavior and is therefore difficult to study. Our laboratory has produced two groups of Sprague–Dawley rats selected for high- (HY) and low-yawning (LY) frequency (Urbá-Holmgren et al., 1990). In previous studies, differences in yawning between HY and LY have been attributed to a possible alteration of the cholinergic or dopaminergic neurotransmission systems (Urbá-Holmgren et al., 1990, 1993). Novelty-induced grooming is often higher in HY than in LY rats (Eguibar and Moyaho, 1997), but little is known yet about the neurochemical mechanisms underlying grooming differences between both groups of rats (Eguibar and Moyaho, 1997). Yawning and grooming are apparently related behaviors since they tend to occur in stress-related contexts (Eguibar and Moyaho, 1997; Moyaho and Valencia, 2002).

In previous studies, it was shown that dopamine D_1 receptors are involved in grooming (Molloy and Waddington, 1987; Van Wimersma Greidanus et al., 1989; Drago et al., 1999), whereas D_2 autoreceptors (Mogilnicka and Klimek, 1977; Yamada and Furukawa, 1980; Urbá-Holmgren et al., 1982; Dourish and Cooper, 1985) or D_2 postsynaptic receptors (Morelli et al., 1986; Serra et al., 1986; Scheel-Krüger, 1986; Ståhle, 1992) bring about yawning. The circumstance that HY and LY rats differ in two behaviors, which distinguish between D_1 and D_2 receptors, led us to investigate to what extent dopamine neurotransmission accounts for the concomitant presence of high numbers of yawns and grooms in HY rats. Specifically, we tested whether HY were more responsive than LY rats to the effect of SKF 38393, a D_1 agonist (Setler et al., 1978), and quinpirole, a D_2 agonist (Tsuruta et al., 1981). We also coadministered single doses of both drugs to test whether the facilitatory role that the stimulation of dopamine D_1 receptors exerts on the behavioral expression of D_2 (Longoni et al., 1987) could explain the difference between the two groups of rats.

2. Methods

2.1. Animals

We used 24 HY and 24 LY male rats which were bred at our laboratory. The HY group was established by recording the yawning of a sample of 2-month-old male rats from

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which a male that yawned 22 times per hour was crossed with one of his sisters. Then he was crossed with his F1 daughters. Afterwards the HY group was maintained by brother–sister mating, selecting HY animals. The LY group was sustained by brother–sister mating, selecting LY animals (Urbá-Holmgren et al., 1990).

After weaning (28 days), the rats were housed in Plexiglas cages (46 × 32 × 20 cm), two to three rats per cage in a colony room with constant temperature (22 ± 1 °C). The rats had free access to food (Lab. Diet, PMI 5008, USA) and tap water and were kept on a 12-h light/dark cycle (lights on at 7:00 a.m.). They were 10–14 weeks old (330 ± 2 g average body weight) when the trials began. To diminish the stress caused by the injections, the rats were handled daily for 8 days before experimentation.

2.2. Drugs

SKF 38393 was dissolved in sterile water as suggested (RBI, USA) and was injected subcutaneously at doses of 0.5, 1, 2, 4, 8, 16, and 32 mg/kg. Quinpirole hydrochloride (RBI) was dissolved in saline (0.9% sodium chloride) and injected intraperitoneally at doses of 25, 50, 100, and 200 μg/kg. The volume of injection of SKF 38393 was 4 ml/kg body weight, while for quinpirole the volume was 1 ml/kg. The coadministration of quinpirole (50 μg/kg) and SKF 38393 (16 mg/kg) followed the procedure used for the individual administration of each drug. The order of injection was randomly assigned. The dosage was chosen because it resulted in the greatest difference in grooming and yawning frequency between the strains. The rats, except those used for the coadministration schedule, were pretreated with vehicle solution.

2.3. Experimental procedure

On trial days, two rats were brought to a room for experiments 30 min before the injections were applied. The room was illuminated with two 60-W fluorescent light lamps. After the injection, the rats were placed in separate compartments in a Plexiglas cage (46 × 32 × 20 cm) that was divided in half with a Plexiglas partition. Yawning and the frequency and duration of grooming episodes were recorded for 90 min using a continuous sampling procedure (Altmann, 1974). A yawn was scored when the rat opened its mouth wide and gradually, maintained the opened position during several seconds, and then closed the mouth rapidly (Urbá-Holmgren et al., 1992). A grooming episode was scored if, as previously described (Gispen and Isaacson, 1981), any of the following components occurred: face washing (vibrating movements of the fore paws in front of the snout, licking of the same paws followed by strokes along the snout, and semicircular movements over the top of the head), body grooming (licking of body fur), genital grooming (licking of genital area), paw licking (licking of fore- and hind-paws), and scratching (scratching of the body with the hind limbs). Interruptions greater than 5 s determined separate grooming episodes.

2.4. Experimental design and statistical analysis

Rats of each group were allocated to each drug treatment: 6 rats for quinpirole, 8 for SKF 38393, and 10 for quinpirole + SKF 38393. Each rat, except those for quinpirole + SKF 38393, received consecutive doses at intervals of 48 h. The data were standardized to control for baseline differences (Zolman, 1993) in grooming and yawning between HY and LY rats, though the untransformed data were used for the graphs. We used multivariate analysis of variance (MANOVA) for repeated measures and the Hotelling–Lawley trace to test for significant effects. We used MANOVA as it does not require the variances of the differences between repeated measures to be homogeneous (Méndez et al., 1994), which is a difficult condition to comply with when using univariate methods (Yandell, 1997). In the case of the coadministration of quinpirole and SKF 38393, two-way ANOVA was used to test for significant effects. Bonferroni *t* test was used in all cases to make multiple comparisons when significant *F*'s were detected. The differences were considered statistically significant when the values of *P* were smaller than .05.

3. Results

3.1. SKF 38393

The number of grooming episodes increased significantly [$F(7,8) = 8.9$, $P < .01$] up to 140% with the highest dose (Fig. 1A). Despite this increase, there was no significant difference between HY and LY rats [$F(1,14) = 0.01$, $P > .05$], and the interaction between the group of rats and drug was not significant [$F(7,8) = 3.3$, $P > .05$]. The mean duration of grooming episodes showed a significant dose-dependent increase followed by a decrease [Fig. 1B; $F(7,8) = 7.3$, $P < .01$]. This effect did not differ between the groups of rats [$F(1,14) = 0.2$, $P > .05$] and the interaction between the group of rats and drug was not significant [$F(7,8) = 1.8$, $P > .05$]. As to yawning, a significant effect was brought about by the drug [Fig. 1C; $F(7,8) = 9.1$, $P < .01$]. Yawning in HY rats decreased up to 91% with the highest dose, while it fluctuated in LY rats. There was also a significant effect for the group of rats [$F(1,14) = 6.2$, $P < .05$], and the interaction between the group of rats and drug was significant, too [$F(7,8) = 13$, $P < .01$], suggesting that SKF 38393 affected yawning differently in HY and LY rats.

3.2. Quinpirole

The number of grooming episodes decreased significantly [$F(4,7) = 15.4$, $P < .01$] with consecutive doses (Fig. 2A). There was a significant difference between the decreases

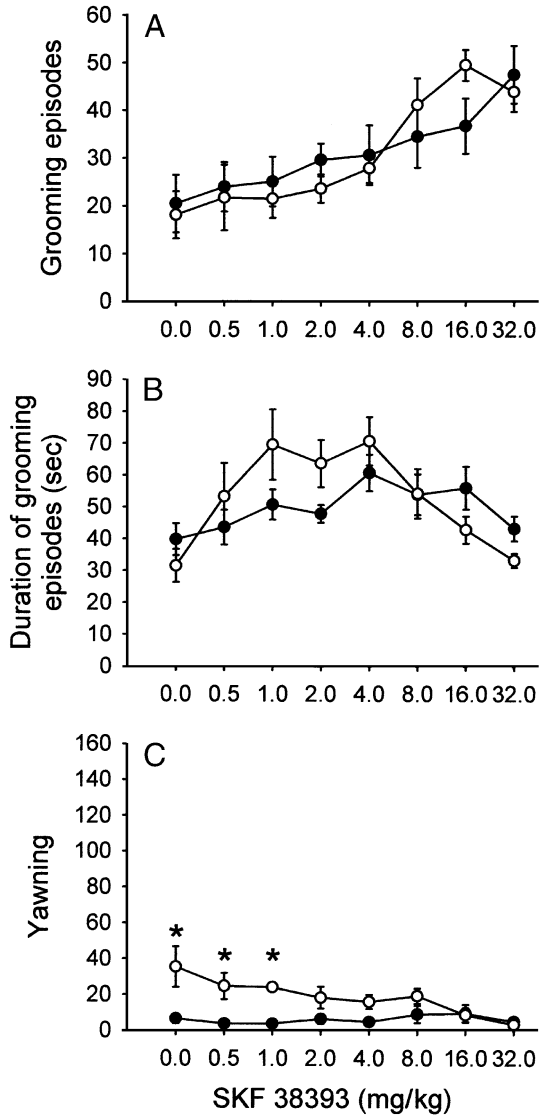

Fig. 1. Effect of SKF 38393 (4 ml/kg wt. sc) on number of grooming episodes (A), duration of grooming episodes (B), and number of yawns (C). The behaviors were recorded for 90 min in LY (filled circles) and HY (open circles) rats. The scores are expressed as mean ± S.E.M. $n = 8$ animals per strain. 0 = vehicle solution alone. * HY and LY rats differed significantly ($P < .05$).

noted for HY and LY rats [$F(1,10) = 12.4$, $P < .01$], and the interaction between the group of rats and drug was not significant [$F(4,7) = 1.2$, $P > .05$]. Duration of grooming episodes declined significantly across consecutive doses [Fig. 2B; $F(4,7) = 6.2$, $P < .01$]. This decline showed no significant difference between HY and LY rats [$F(1,10) = 0.3$, $P > .05$], and the interaction between the group of rats and drug was not significant [$F(4,7) = 0.8$, $P > .05$]. In the case of yawning, there was a significant effect of the drug [$F(4,7) = 37.1$, $P < .01$] with an initial 13-fold increase followed by a decline (Fig. 2C). The effect was significantly greater in HY than in LY rats [$F(1,10) = 22.1$, $P < .01$], and the interaction between the group of rats and drug was statistically significant as well [$F(4,7) = 12.9$, $P < .01$], showing that the effect of the drug on yawning differed between the groups of rats.

3.3. Quinpirole+SKF 38393

Quinpirole (50 μg/kg) and SKF 38393 (16 mg/kg) coadministered had no effect on the mean number of grooming episodes [Fig. 3A; $F(1,16) = 1$, $P > .05$]. In LY rats, the number of episodes did not vary relative to vehicle-treated rats, while in HY rats there was an increase of up to 36%, although this did not result in an overall significance. It can be observed that in rats given 16 mg/kg of SKF 38393 alone, grooming episodes increased on average 54% in both groups (Fig. 1A). In rats given quinpirole + SKF 38393, the mean

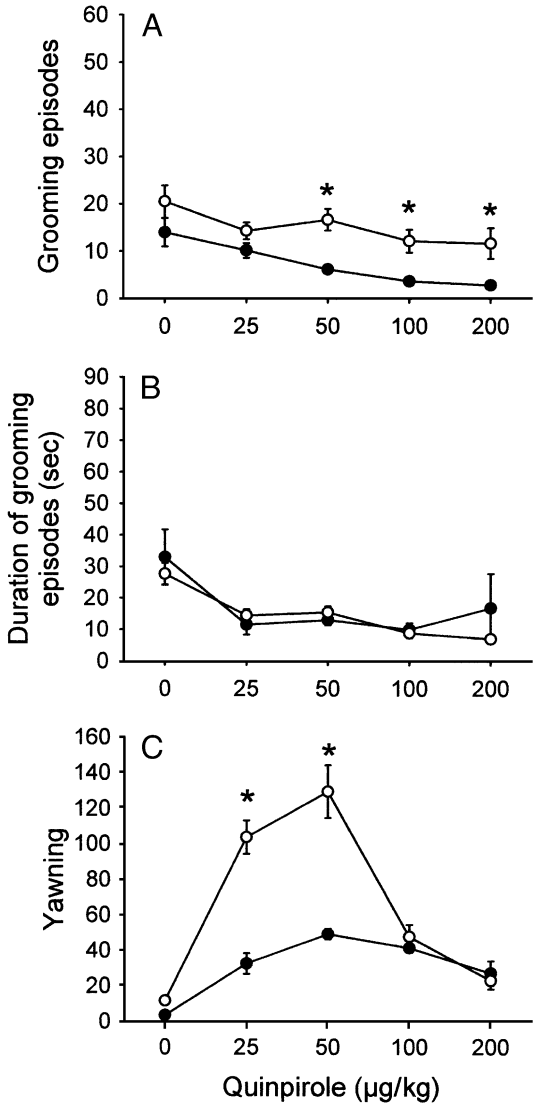

Fig. 2. Effect of quinpirole (1 ml/kg wt. ip) on number of grooming episodes (A), duration of grooming episodes (B), and number of yawns (C). The behaviors were recorded for 90 min in LY (filled circles) and HY (open circles) rats. The scores are expressed as mean ± S.E.M. $n = 6$ animals per strain. 0 = vehicle solution alone. * HY and LY rats differed significantly ($P < .05$).

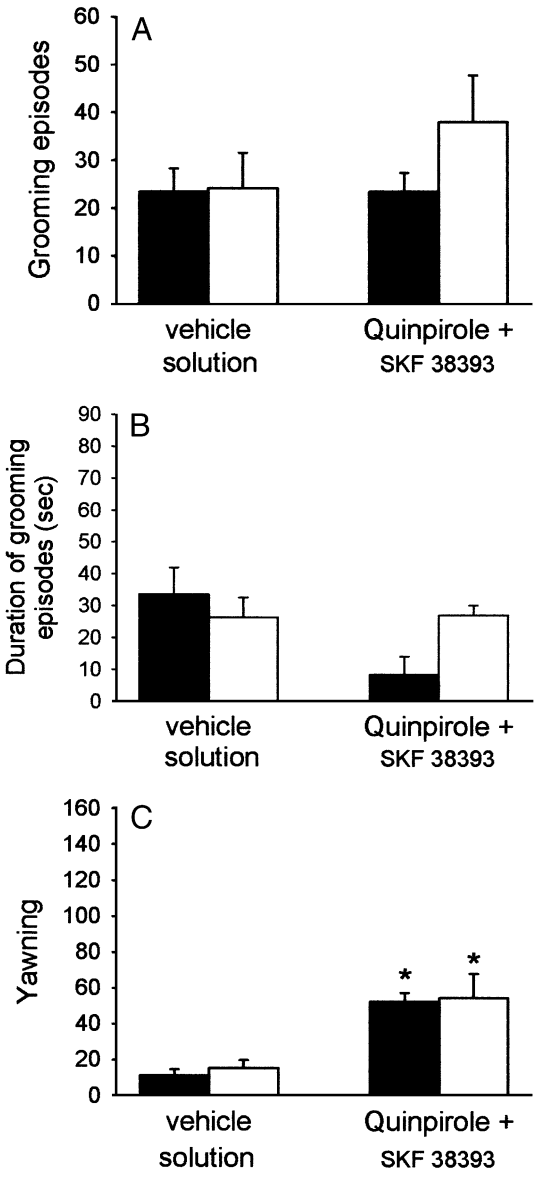

Fig. 3. Effect of 16 mg/kg of SKF 38393 + 50 μg/kg of quinpirole on number of grooming episodes (A), duration of grooming episodes (B), and number of yawns (C). The behaviors were recorded for 90 min in LY (filled bars) and HY (open bars) rats. The scores are expressed as mean ± S.E.M. $n = 10$ animals per strain. * HY and LY differed significantly ($P < .05$) from their vehicle solution groups.

duration of grooming episodes decreased 16% in LY rats and increased 2% in HY rats relative to vehicle-treated rats (Fig. 3B), though these changes were not statistically significant [$F(1,16) = 0.2$, $P > .05$]. In contrast, in HY and LY rats given 16 mg/kg of SKF 38393 alone, the mean duration of grooming episodes increased about 39% (Fig. 1B). When quinpirole and SKF 38393 were coadministered, LY rats yawned only 4.6 times more than vehicle-treated rats. Yawning among HY rats increased 3.5 times. Although these increments were significant relative to vehicle-treated rats [Fig. 3C; $F(1,16) = 26.7$, $P < .01$], the effect showed no difference between HY and LY rats [$F(1,16) = 0.2$, $P > .05$]. It can be noted that rats given 50 μg/kg of quinpirole yawned about 13 times more than vehicle-treated rats (Fig. 2C).

4. Discussion

Although we applied consecutive doses of quinpirole and SKF 38393 to the same subjects, accumulative effects on the measured behaviors are unlikely since 48 h elapsed between doses. In addition, it is known that SKF 38393 does not generate behavioral tolerance (Neisewander et al., 1991). Moreover, the dose–response curves of yawning and grooming do not fit the prediction that greater doses would be necessary to reinstate the initial effect (Carlton, 1983).

The increment in the number and duration of grooming episodes with SKF 38393 in HY and LY rats is consistent with previous studies on other strains of rats (Braun and Chase, 1987; Longoni et al., 1987; Molloy and Waddington, 1987; Serra et al., 1990), and accords with the view that D_1 receptor activation contributes to the initiation as well as the completion of grooming sequences (Berridge and Aldridge, 2000). The finding that HY and LY rats did not differ in the number or duration of grooming episodes in response to SKF 38393 suggests that dopamine D_1 receptors do not play a direct role in grooming between these groups of rats. In contrast, the fact that quinpirole did not inhibit grooming episodes in HY rats as much as in LY rats suggests that D_2 receptor activation can produce differences in grooming between the two groups of rats. Since the difference was restricted to the frequency of grooming episodes, it appears that quinpirole can distinguish between grooming frequency and duration. Although the decrease in grooming episodes with quinpirole is consistent with previous studies (White et al., 1988; Eilam and Szechtman, 1989; Eilam et al., 1989, 1992; Jackson et al., 1989, but see Braun and Chase, 1987; Walters et al., 1987), there are no previous reports, as far as we know, in which quinpirole can affect differentially the frequency and duration of grooming episodes.

The increment in yawning with quinpirole agrees with previous reports (Longoni et al., 1987; Spina et al., 1989; Kostrzewa and Brus, 1991). Similarly, the direction of the difference in yawning between HY and LY rats after the administration of quinpirole accords with studies that indicate that apomorphine and (−)-3-PPP have a greater effect on HY than on LY rats (Urbá-Holmgren et al., 1993). In the present study, HY and LY rats differed in yawning in response to low doses of quinpirole which primarily affect DA autoreceptors (Di Chiara et al., 1978). This agrees with the suggestion that yawning is an autoreceptor-mediated response, although D_1 receptor activation may also be involved, as the inhibitory effect of SKF 38393 on yawning differed between HY and LY rats. In fact other studies have queried the hypothesis that yawning is an autoreceptor-mediated response (Ståhle and Ungerstedt, 1986), and some authors have provided suggestive evidence for a role of dopamine D_3 receptors in eliciting yawning (Kostrzewa and

Brus, 1991; Damsma et al., 1993). Therefore, there is the possibility that differences in yawning frequency between HY and LY rats are the result of quinpirole activating D_3 receptors. On the other hand, there is evidence that dopamine D_1 and D_2 receptors are coupled (Arnt, 1985a,b), and that the stimulation of D_1 receptors by endogenous dopamine exerts a facilitatory role in the behavioral expression of D_2 receptor activation (Morelli et al., 1986; Braun and Chase, 1987; Longoni et al., 1987). According to this hypothesis, the coadministration of quinpirole and SKF 38393 would potentiate yawning. However, the results did not corroborate this prediction, as yawning was below the level reached when quinpirole alone was administered. Nor did the results agree with earlier studies which indicate that D_1 receptor activation inhibits yawning induced by apomorphine (Zarkovsky and Cereska, 1989) or by bromocriptine (Canales and Iversen, 2000), a D_2-class receptor agonist. Instead, our findings indicate that when coadministered, the effect of quinpirole acted against SKF 38393.

In summary, the results presented here indicate that the activation of D_2 receptors contributes to a greater extent than is the case for D_1 receptors to yawning and grooming differences between HY and LY rats.

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