Behavioral Neuroscience
2006, Vol. 120, No. 5, 1084–1092

0735-7044/06/$12.00 DOI: 10.1037/0735-7044.120.5.1084

Differential Responsiveness to Fluoxetine During Puberty

Kereshmeh Taravosh-Lahn, Christel Bastida, and Yvon Delville
University of Texas at Austin

In male golden hamsters (*Mesocricetus auratus*), attack frequency decreases during puberty. As serotonin inhibits offensive responses in adult hamsters, it is hypothesized that the serotonin system becomes upregulated in the hypothalamus during puberty. This hypothesis was tested through acute treatment with fluoxetine, a serotonin reuptake inhibitor, as well as through analysis of serotonin innervation in specific brain areas. In adults, fluoxetine treatment inhibited aggressive behavior. In juveniles, high doses of fluoxetine only reduced offensive responses (i.e., frequency and repetition of attacks), whereas low doses enhanced them. Juveniles also showed a dose-specific maturation of attack targets. In addition, the density of serotonin innervation of the hypothalamus was 20% higher in adult hamsters compared with juveniles. On the basis of these data, it is proposed that the developing serotonergic system shapes the development of offensive behaviors in male golden hamsters.

Keywords: aggression, play fighting, adolescence, serotonin, SSRI

In hamsters, play-fighting behavior changes gradually into adult aggression during puberty (Goldman & Swanson, 1975; Wommack, Taravosh-Lahn, David, & Delville, 2003; reviewed in Delville, Newman, Wommack, Taravosh-Lahn, & Cervantes, 2005). In males, this time period is marked by an increase in testosterone levels, testicular development, and the emergence of sexual behaviors (Goldman & Swanson, 1975; Miller, Whitsett, Vandenbergh, & Colby, 1977; Vomachka & Greenwald, 1979; Wommack, Salinas, Melloni, & Delville, 2004). On the basis of these variables, puberty can be divided into three categories: early, mid, and late. During early puberty (Postnatal Days 30–40), hamsters engage in play fighting but not sexual behavior. Testes are small, and testosterone levels begin their rise. Midpuberty (Postnatal Days 40–50) is marked by the onset of sexual behavior, elevated testosterone, and moderately developed testes. This is also a period of transition from play fighting to adult aggression. By late puberty (Postnatal Days 50–60), testosterone levels and testes are fully mature, and animals start engaging in adult aggression.

Three different aspects of offensive responses have been shown to undergo changes during puberty in male hamsters. The frequency of attacks during agonistic encounters peaks around Postnatal Day 35 (P-35) and decreases into late puberty and early adulthood (Postnatal Day 70 [P-70]; Goldman & Swanson, 1975; Pellis & Pellis, 1988; Taravosh-Lahn & Delville, 2004; Wommack et al., 2003). Furthermore, the areas on the body of the protagonists initially targeted during attacks also undergo a gradual transition during this period (Taravosh-Lahn & Delville, 2004; Wommack et al., 2003). In early puberty, during the peak of play-fighting activity, attacks are predominantly directed at the cheeks and face of the opponents (play-fighting attacks). By midpuberty, the hamsters direct their attacks toward the flanks of their opponents (side attacks). By late puberty, attacks directed at the lower belly and rump become dominant (adult attacks) and persist throughout adulthood.

In addition, a recent analysis of the temporal distribution of attacks during encounters has shown further changes during puberty (Cervantes, Taravosh-Lahn, Wommack, & Delville, in press). One notable aspect of this study relates to the repetition of attacks during bouts of contact with an opponent. During the peak of play-fighting activity on P-35, attacks are highly repetitive within the same bout of contact. At this time, the hamsters continuously engage in play fighting. By early adulthood, the repetition of attacks per bout decreases. Typically, adult males will initiate an attack on intruders, bite them, then rest for some time before resuming contact and another attack (Cervantes et al., in press). Together, these data provide a more complex description of the peri-pubertal maturation of offensive responses during agonistic encounters in hamsters.

However, the neural mechanisms underlying these behavioral changes remain poorly understood. Although the neurobiology of offensive aggression has been extensively studied in adult male hamsters, little is known about the control of play-fighting behavior (Delville et al., 2005). In adult males, offensive responses are modulated by an interaction between vasopressin and serotonin in the hypothalamus (Delville, Mansour, & Ferris, 1996a; Ferris et al., 1997; Ferris, Stolberg, & Delville, 1999). Vasopressin facilitates the behavior, whereas serotonin inhibits it. As serotonin release in the hypothalamus inhibits offensive aggression in adult hamsters (Ferris et al., 1999), it is possible that the decreased

Kereshmeh Taravosh-Lahn, Christel Bastida, and Yvon Delville, Institute for Neuroscience and Psychology Department, University of Texas at Austin.

This study was supported by National Science Foundation Grant IOB 0516272 and by National Institutes of Health Grant ES10385 awarded to Yvon Delville, as well as a University of Texas Continuing Fellowship awarded to Kereshmeh Taravosh-Lahn. We thank Chatainya Indukuri for expert technical assistance and Joel Wommack, Catalina Cervantes, and Kris Singletary for insightful comments on the study.

Correspondence concerning this article should be addressed to Kereshmeh Taravosh-Lahn, Institute for Neuroscience and Psychology Department, University of Texas at Austin, 1 University Station, A8000, Austin, TX 78712. E-mail: kereshmeh@mail.utexas.edu

frequency and repetition of attacks during puberty is associated with increased serotonin availability within the hypothalamus. A single neural circuitry involving vasopressin and serotonin would control the maturation of offensive responses in hamsters.

This possibility was addressed in the present study. First, we compared responsiveness to peripheral treatment with fluoxetine, a serotonin reuptake inhibitor, between adult and juvenile male hamsters. Fluoxetine inhibits aggression in adult male hamsters (Delville et al., 1996a, Ferris et al., 1997). We hypothesized that the inhibitory effect of fluoxetine on offensive responses would be more pronounced in adults compared with juveniles. Therefore, we compared hamsters at the two developmental time points with the highest and lowest frequencies of offensive responses. Second, we compared the density of serotonin innervation of the limbic areas associated with aggression in adults, such as the anterior hypothalamus (AH) and ventrolateral hypothalamus (VLH; Delville, De Vries, & Ferris, 2000; Delville, Mansour, & Ferris, 1996b; Ferris et al., 1999).

Method

Subjects and Treatment

Male golden hamsters (*Mesocricetus auratus*) were bred in a colony maintained in the laboratory and founded with animals purchased from Harlan Sprague Dawley (Indianapolis, IN). Shortly after birth, litters were culled to 6 pups containing both males and females. All hamsters were weaned on Postnatal Day 25 and were individually housed in Plexiglass cages (8 inches × 13 inches × 5 inches or 20.32 cm × 33.02 cm × 12.70 cm), as golden hamsters are solitary animals in the wild (Weinart, Fritzsche, & Gattermann, 2001). Food and water were provided ad libitum. All hamsters used in the experiments were housed under a reversed daylight cycle (14:10-hr light–dark cycle; lights on at 2000). All experimental procedures were performed according to National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee of the University of Texas at Austin, an Association for Assessment of Laboratory Animal Care approved facility.

Experimental Design

The developmental effects of fluoxetine administration were examined by using hamsters either during early puberty (P-35) or early adulthood (P-70). These time points were selected for comparison as attack frequency peaks during early puberty (P-35) and subsequently declines into early adulthood (P-70). All hamsters were experienced with agonistic encounters through repeated exposure to intruders in their home cage several times during development. This prior exposure to intruders ensured that all subjects had sufficient social experience and would indeed engage in offensive responses. On the test days, the hamsters received an intraperitoneal injection of fluoxetine hydrochloride (0 mg/kg, 10 mg/kg, or 20 mg/kg; dissolved in 20% dimethyl sulfoxide in saline [vol/vol]; Sigma Chemical, St. Louis, MO). These doses of fluoxetine were based on prior studies in hamsters (Ferris et al., 1997). The volumes injected ranged from 0.18 mL to 0.21 mL. Separate hamsters were used for each dose and each test day (P-35: 0 mg/kg, $n = 9$, 10 mg/kg, $n = 13$, 20 mg/kg, $n = 10$; P-70: 0 mg/kg, $n = 7$, 10 mg/kg, $n = 10$, 20 mg/kg, $n = 9$). Two hours after injection, the hamsters were exposed to a smaller (10%–20%) and younger (5–10 days) same-sex conspecific intruder in their home cage for a 10-min period. The 2-hr period between injection and behavioral testing was optimized in previous studies (Ferris et al., 1997). The encounters were videotaped for later review with iMovie (Apple Computers, Cupertino, CA).

Immediately after the resident–intruder test, the hamsters were placed in a Lat maze for another 10-min period to test for locomotor activity. The eponymous Lat-maze apparatus was named after the pre-Islamic goddess al-Lat who was worshipped by Arabs using a sacred stone cube. The Lat maze consists of an open-faced square box (63.0 cm × 63.0 cm × 21.5 cm) containing a smaller closed square box (39 cm × 39 cm × 17.5 cm) at its center. The resulting space between the two boxes forms a corridor that is marked with a line every 12 cm (Cervantes, David, Loyd, Salinas, & Delville, 2005). These lines drawn across the corridor permit a quantification of locomotor activity. The number of lines crossed was counted for the 10-min period. All tests were conducted during the first half of the dark phase.

Descriptions of Agonistic Behaviors

Several behaviors were observed during resident–intruder testing. These behaviors included attacks, pins, contact time, contact bouts, attack latency, and target of attack. All behaviors recorded were performed by the resident hamster in response to the presence of an intruder. During the test period, the resident hamster would approach and contact the intruder several times, each of these times being referred to as a *contact bout* (unit of interaction). A contact bout was recorded for each time the resident initiated contact with the intruder for at least 5 s and ended when contact ceased. Contact time was recorded for the total duration of the testing period during which the resident initiated and maintained contact with the intruder. An attack consisted of a combination of approach followed immediately by an attempt to bite. Attacks per bout were calculated by dividing the total number of attacks by the total number of contact bouts that occurred during the testing period. Pins were recorded when the resident would restrain the intruder on its back by pinning it with its forepaws. Attack latency was recorded as the duration of time elapsed before the first attack occurred during the testing period. During an attack, the location on the body of the intruder that the resident attempted to bite was referred to as the target of attack. These targets were divided into three categories: play-fighting, side, and adult attacks. An attack initially aimed at the cheek and face of the intruder was recorded as a play-fighting attack. Attacks targeting the flanks or dorsolateral portion of the intruder's torso were recorded as side attacks. Attacks aimed at the rump or lower belly were recorded as adult attacks. The percentage of each attack type was calculated by dividing each of the three categories of attack targets by the total number of attacks. The final bite location was not considered the target of attack because bites are often redirected by the defensive behavior of the intruder.

Serotonin Innervation

A second group of hamsters was sacrificed at either P-35 ($n = 7$) or P-70 ($n = 13$), and their brains were processed for immunocytochemistry to serotonin as previously described (Delville, Melloni, & Ferris, 1998). Briefly, hamsters were perfused under anesthesia with saline followed by 4% paraformaldehyde in 0.1-M phosphate-buffered saline (wt/vol). Brains were sliced into 40-μm thick sections after brief postfixation and storage in 20% sucrose in phosphate-buffered saline (wt/vol) at 4 °C overnight. Sections were labeled with an antibody to serotonin (1/7500; Protos Biotech, New York, NY) after successive incubations in a secondary antibody (goat anti-rabbit, biotinylated; Vector Labs, Burlingame, CA), a tertiary complex (Vectastain Avidin Biotin Complex Elite Kit, Vector Labs), and nickel-conjugated 3,3-diaminobenzidine tetrahydrochlorideβ.

The density of serotonin-immunoreactive varicosities was quantified with National Institutes of Health Image Software (Version 1.62) in various parts of the brain associated with agonistic behavior (David, Cervantes, Trosky, Salinas, & Delville, 2004; Delville et al., 2000), as previously described (Delville et al., 1998). Images were captured via a video camera mounted on the microscope at 100× and digitized, using changes in gain to normalize background intensities to a standard grayscale value. The

areas selected for quantification included the AH just ventrolateral to the nucleus circularis, the VLH including the medial aspects of the medial tubercular nucleus and the ventrolateral part of the ventromedial hypothalamic nucleus, the posterodorsal part of the medial amygdala (MePD), and the lateral septal nucleus at the juncture of the intermediate and ventral aspects. The values used for each hamster represented the average of several successive sections across these areas. The density of serotonin-immunoreactive varicosities was taken from samples (150-μm circles) at the center of these areas (David et al., 2004; Delville et al., 2000).

Data Analysis

Parametric data (percentages, durations) were analyzed through two-way analyses of variance (ANOVAs; independent variables were age and treatment). One-way ANOVAs were also used as planned comparisons to analyze the effect of dose at each age group. Nonparametric data (frequencies, numbers of lines) were analyzed through separate Kruskal–Wallis tests followed by Mann–Whitney tests for each test day (two-tailed). Serotonin immunoreactivity was compared between ages using separate Student's t tests for each brain area.

Results

Behavioral Observations

Juvenile hamsters responded to fluoxetine differently than adults. In adults, fluoxetine caused a general inhibition of agonistic behavior including attack frequency and contact time. Juveniles, however, responded differentially to the high and low doses of fluoxetine. The low dose resulted in an activation of agonistic behaviors including attack frequency, attacks per bout, and contact time. Unlike adults, the high dose resulted in only a partial inhibition of agonistic behavior. Attack targets were also affected in juveniles.

Hamsters tested on P-70 showed a decrease in attacks in response to increasing doses of fluoxetine, $H(2) = 11.0$, $p < .01$ (Kruskal–Wallis test; see Figure 1). Attacks were almost entirely inhibited at the high dose ($U = 5.5$, $U' = 57.5$, $p < .01$, Mann–Whitney test). There was no effect of fluoxetine on attacks at the low dose ($U = 16.5$, $U' = 46.5$, $p > .1$). However, on P-35, the effects of fluoxetine treatment on attacks varied depending on the doses administered, $H(2) = 11.2$, $p < .01$. High doses of fluoxetine resulted in only a partial reduction of offensive responses ($U = 25$, $U' = 65$, $p > .1$). However, low doses led to a significant activation of offensive responses ($U = 28.5$, $U' = 88.5$, $p < .05$).

Attack latencies were first analyzed through a two-way ANOVA (see Figure 1). Overall, hamsters tested on P-70 were slower to attack their intruder than on P-35, $F(1, 49) = 18.5$, $p < .001$. In addition, there was an overall effect of the fluoxetine dose as increasing doses delayed the onset of attacks, $F(2, 49) = 14.0$, $p < .0001$. However the interaction between the two independent variables was a trend and was not statistically significant, $F(2, 49) = 2.8$, $p = .07$. In addition, planned analyses were performed for each age. Hamsters tested on P-70 exhibited an increase in attack latency in proportion to increasing fluoxetine dosage, $F(2, 20) = 7.5$, $p < .01$. Adults given the high dose had significantly higher attack latencies than vehicle-treated hamsters ($p < .001$, Fisher's probable least-squares difference test). Low-dose adults also had significantly higher attack latencies when compared with vehicle-treated hamsters ($p < .05$, Fisher's probable least-squares difference test). On P-35, there was also a significant effect of dose on attack latency, $F(2, 29) = 7.727$, $p < .01$. There was no effect of the low dose of fluoxetine on attack latency when compared with vehicle-treated hamsters ($p > .1$). However, there was a significant increase in attack latency at the high dose in juveniles ($p < .01$).

In adults, there was an overall effect of fluoxetine dose on the number of pins, $H(2) = 10.2$, $p < .01$ (see Figure 1). Low-dose hamsters performed significantly fewer pins compared with vehicle hamsters ($U = 13$, $U' = 43$, $p < .05$). Similarly, high-dose hamsters also performed significantly fewer pins in comparison with vehicle-treated hamsters ($U = 9$, $U' = 54$, $p < .01$). There was also an overall effect of fluoxetine on pins in juvenile hamsters, $H(2) = 9.8$, $p < .01$. In juveniles tested at the low dose, there was no effect of fluoxetine on the number of pins when compared with vehicle-treated hamsters ($U = 39.5$, $U' = 77.5$, $p > .1$). Juveniles tested at the high dose showed an inhibition of pins when compared with vehicle hamsters ($U = 19$, $U' = 71$, $p < .05$).

Contact-time duration was significantly lower in adults than in juveniles, $F(1, 50) = 22.12$, $p < .001$ (see Figure 2). There was also an overall significant effect of fluoxetine on contact time, $F(2, 50) = 9.15$, $p < .001$. However, the interaction between independent variables was not significant, $F(2, 50) = .860$, $p > .1$. In adults, fluoxetine treatment resulted in an increasing reduction of contact time in direct proportion to increasing dosage, $F(2, 21) = 4.484$, $p < .05$. Adults given the high dose showed a significant decrease in contact time when compared with vehicle-treated hamsters ($p < .05$). Low-dose hamsters showed a partial reduction, but this difference was not significant ($p > .1$). In juveniles, contact time for neither the high nor low dose differed significantly from vehicle-treated hamsters ($p > .1$).

The number of contact bouts performed by juveniles was slightly affected by drug treatment, but these changes only showed a trend, $H(2) = 5.7$, $p = .0581$ (see Figure 2). In adults, fluoxetine did have an effect on contact bout frequency ($p < .01$). Adult hamsters given the high dose engaged in fewer contact bouts than vehicle-treated hamsters ($U = 5$, $U' = 58$, $p < .01$). There was no effect of contact bout frequency in low-dose hamsters in comparison with vehicle-treated hamsters ($p > .1$).

There was a decrease in attacks per bout in adults when compared with juveniles, $F(1, 50) = 9.845$, $p < .01$ (see Figure 2). There was also a significant effect of fluoxetine dose on the number of attacks per bout, $F(2, 50) = 5.454$, $p < .01$. The interaction between the independent variables (age and treatment) was statistically significant, $F(2, 50) = 4.599$, $p < .05$. In adults, there was a trend towards a decrease in attacks per bout in response to fluoxetine, $F(2, 22) = 3.295$, $p = .056$. In juveniles, there was an overall effect of fluoxetine on attacks per bout, $F(2, 28) = 7.984$, $p < .01$. In these hamsters, the number of attacks per bout was significantly higher at the low dose when compared with vehicle-treated hamsters ($p < .05$). However, high-dose hamsters did not perform differently from vehicle-treated hamsters at P-35 ($p > .1$).

Hamsters at P-35 perform either play-fighting attacks or side attacks, whereas P-70 hamsters perform only adult attacks (see Figure 3). The pattern of attack targets did not change in P-70 hamsters, as this behavior had already matured (100% adult attacks). The target of attacks changed in a dose-specific manner in P-35 hamsters, $F(2, 26) = 6.292$, $p < .01$. The percentage

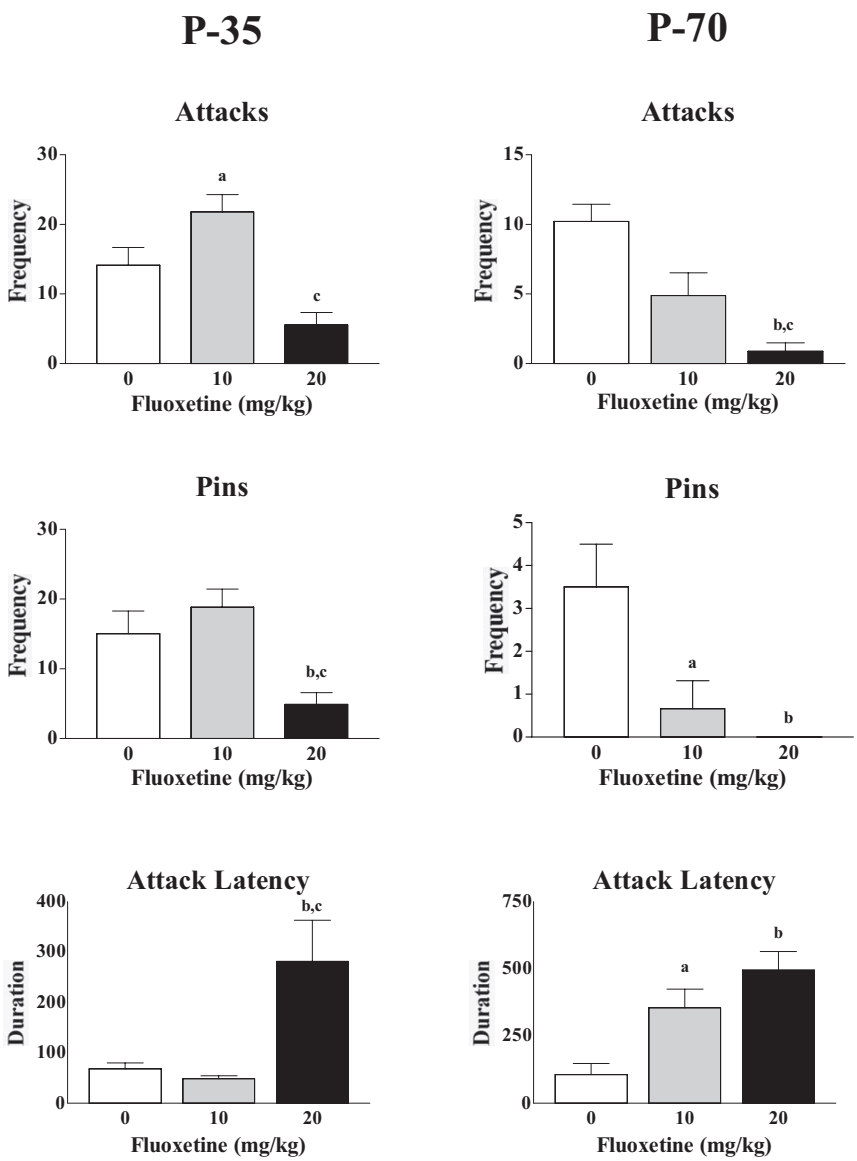

Figure 1. Comparison of attack frequency, pin frequency, and attack latency observed during a 10-min resident–intruder test at different doses of fluoxetine between Postnatal Day 35 (P-35) and Postnatal Day 75 (P-75) male golden hamsters. a = statistical differences between the 0- and 10-mg/kg dose of fluoxetine, b = statistical differences between the 0- and 20-mg/kg dose of fluoxetine, and c = statistical differences between the 10- and 20-mg/kg dose of fluoxetine, with $p < .05$, at least. Error bars represent standard error.

of play-fighting attacks decreased in response to fluoxetine treatment. Both low- and high-dose juveniles performed significantly fewer play-fighting attacks than vehicle-treated hamsters ($p < .01$). Similarly, side attacks were also affected by fluoxetine, $F(2, 26) = 4.739$, $p < .05$. There was a significant increase in side attacks at the low dose when compared with vehicle-treated hamsters ($p < .01$). High-dose hamsters also showed an increase in side attacks, but this difference was not statistically significant ($p > .1$). All hamsters tested at P-35 performed less than 5% adult attacks, with no significant difference between groups.

The analysis of locomotor activity was important to further determine the selectivity of the effects of fluoxetine on agonistic behavior. The average number of lines crossed in a Lat maze was not affected by fluoxetine in juvenile hamsters, $H(2) = 1.66$, $p > .1$ (see Figure 4). However, fluoxetine did have an effect in the number of lines crossed in adults, $H(2) = 6.56$, $p < .05$. There was a significant decrease in the number of lines crossed by hamsters given the high dose of fluoxetine at P-70 when compared with vehicle-treated hamsters ($U = 6$, $U' = 44$, $p < .05$). Low-dose hamsters did not perform differently than vehicle-treated hamsters ($U = 19$, $U' = 35$, $p > .1$).

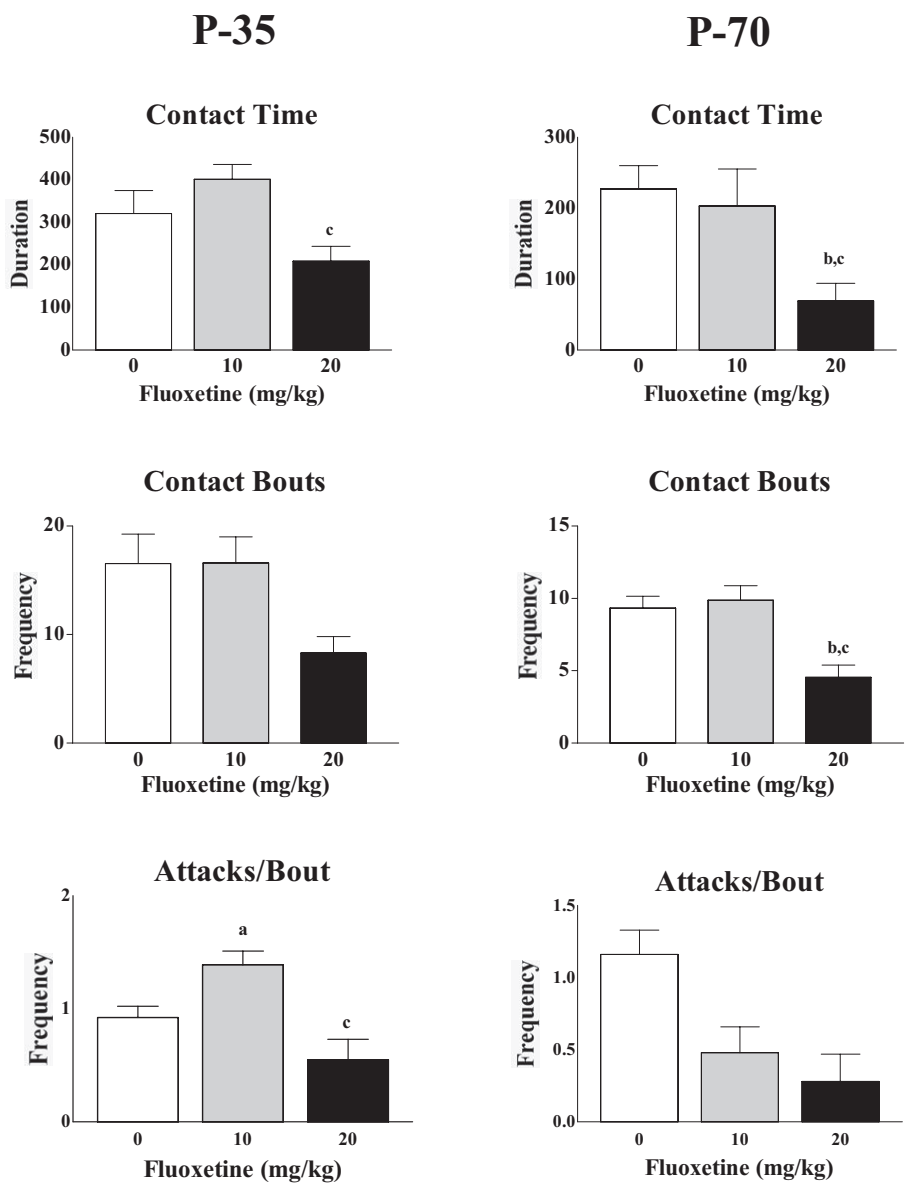

Figure 2. Comparison of contact time duration, frequency of contact bouts, and number of attacks per bout observed during a 10-min resident–intruder test at different doses of fluoxetine between Postnatal Day 35 (P-35) and Postnatal Day 75 (P-75) male golden hamsters. a = statistical differences between the 0- and 10-mg/kg dose of fluoxetine, b = statistical differences between the 0- and 20-mg/kg dose of fluoxetine, and c = statistical differences between the 10- and 20-mg/kg dose of fluoxetine, with $p < .05$, at least. Error bars represent standard error.

Serotonin Innervation

Serotonin immunoreactive fibers as well as varicosities were seen throughout sections in all selected areas. The density of serotonin-immunoreactive varicosities differed between age groups, but only in specific areas (see Figure 5). There was no difference in serotonin-immunoreactive varicosities within the VLH, $t(9) = 2.262$, $p > .1$, and the lateral septum, $t(10) = 2.228$, $p > .1$, between P-35 and P-70. However, there was a 20% increase in the density of serotonin-immunoreactive varicosities in adults compared with juveniles in the AH, $t(18) = 2.101$, $p < .01$, and the MePD, $t(10) = 2.228$, $p < .05$, from P-35 to P-70.

Discussion

Offensive responses in hamsters undergo several changes during the course of development (Cervantes, Taravosh-Lahn, & Delville, 2005; Taravosh-Lahn & Delville, 2004; Wommack et al., 2003). On the basis of these observations, it was hypothesized that juvenile hamsters would be less responsive to serotonin than adults.

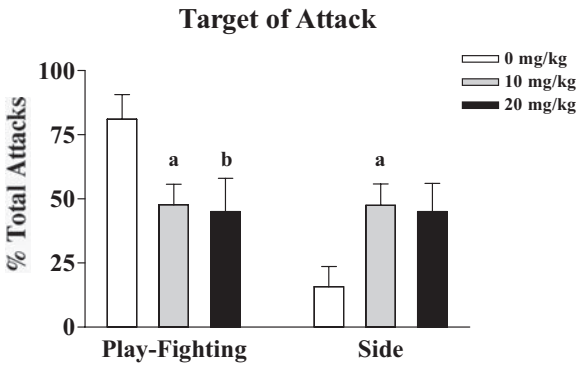

Figure 3. Comparison of attack targets (play fighting and side attacks) observed during a 10-min resident–intruder test at different doses of fluoxetine in Postnatal Day 35 (P-35) male golden hamsters. a = statistical differences between the 0- and 10-mg/kg dose of fluoxetine, and b = statistical differences between the 0- and 20-mg/kg dose of fluoxetine, with $p < .05$, at least. Error bars represent standard error.

The data partially support this hypothesis. In adults, treatment with fluoxetine resulted in an inhibition of offensive responses directly proportionate to increasing doses, consistent with previous observations in this species (Delville et al., 1996a; Ferris et al., 1997; Grimes & Melloni, 2002). Unexpectedly, the effects of fluoxetine on juveniles were rather different. At elevated doses, fluoxetine caused only a partial reduction of offensive responses in younger animals. It is interesting to note that lower doses of fluoxetine resulted in an increase in attack frequency in juvenile hamsters. Furthermore, the decreased responsiveness to elevated doses of fluoxetine was paralleled by the quantification of serotonin innervation in the brain. Serotonin innervation increases during puberty within neural sites associated with offensive aggression in adult hamsters (David et al., 2004; Delville et al., 2000; Ferris et al., 1997). Consequently, our data at least partially support the hypothesis that the decrease in attack frequency occurring during puberty is associated with an increase in serotonin availability within the neural network controlling offensive responding. Furthermore, these data are also consistent with the hypothesis that a single neural circuitry, centered on the AH, controls offensive responses in golden hamsters across peri-pubertal development (Delville, David, Taravosh-Lahn, & Wommack, 2003).

In addition, a differential response to acute doses of fluoxetine was observed in juvenile hamsters. Low doses enhanced offensive responses, whereas high doses resulted in a partial inhibition. In adult hamsters, treatment with fluoxetine resulted in an inhibition of offensive responses proportionate to increasing dosage. Treatment with low doses of fluoxetine led to a reduction in attacks, attacks per bout, and pin frequency and led to an increase in attack latency, though only pins and attack latencies were statistically significant. At the high doses of fluoxetine, attacks, pin frequency, and attack latencies were strongly affected and were statistically significant from controls. In contrast, the inhibition of offensive responses in juveniles was rather limited. Treatment with high doses of fluoxetine only reduced pin frequency and increased attack latency but had no significant effect on attack frequency or attacks per bout. Moreover, low doses enhanced offensive responses. This effect was apparent with attacks per bout and attack frequencies but not with pin frequencies or attack latencies. Together, these data show that the effects of fluoxetine on offensive responses are both age and dose specific.

The effects of fluoxetine on attack frequency could possibly be explained through changes in attack repetition during contact bouts, changes in contact bout numbers, or a combination of both. The overall increase in attack frequency at low doses of fluoxetine in juveniles was associated with an increased repetition of attacks per bout rather than a change in number of contact bouts. These data show that low doses of fluoxetine in juveniles selectively affected the frequency of attacks per bout during testing. It is interesting to note that, in adults, high doses of fluoxetine inhibited both the overall attack frequency as well as contact bout number and, consequently, attacks per bout. These data suggest that the nature of the effects of fluoxetine on overall attack frequency differs between low doses in juveniles and high doses in adults.

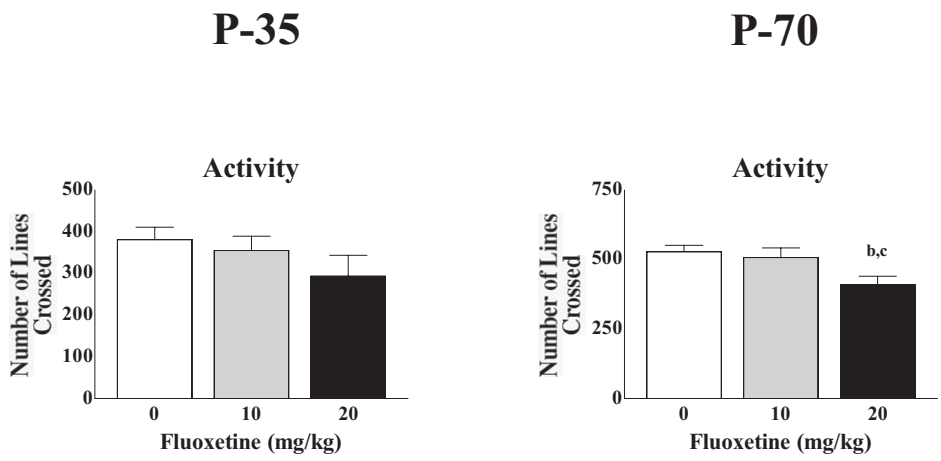

Figure 4. Comparison of activity levels observed during a 10-min Lat maze test at different doses of fluoxetine between Postnatal Day 35 (P-35) and Postnatal Day 70 (P-70) male golden hamsters. b = statistical differences between the 0- and 20-mg/kg dose of fluoxetine, and c = statistical differences between the 10- and 20-mg/kg dose of fluoxetine, with $p < .05$, at least. Error bars represent standard error.

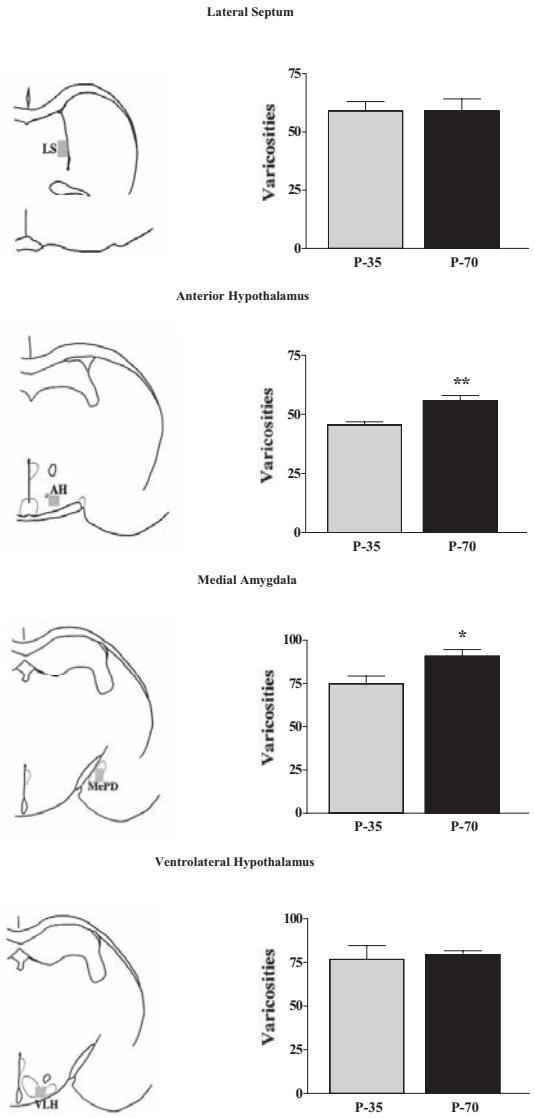

Figure 5. Comparison of the density of serotonin-immunoreactive varicosities within the anterior hypothalamus, lateral septum, medial amygdala, and the posterodorsal part of the ventrolateral hypothalamus in Postnatal Day 35 (P-35) and Postnatal Day 70 (P-70) male golden hamsters. Varicosities were counted within standard surfaces placed over digitized images. Representative coronal sections of the areas of interest were drawn using a camera lucida attachment on a microscope. Error bars represent standard error. $*p < .05$, $**p < .01$.

In addition, the increased repetition of attack per bout in juveniles may be discussed in the context of changes in the development of agonistic behavior during puberty. In male hamsters, the repetition of attacks decreases from a peak in early puberty, as the hamsters play fight, to a minimum in early adulthood (Cervantes et al., in press). In this context, attack repetition is an index of play fighting in hamsters. Thus, it could be argued that in juveniles, low doses of fluoxetine enhance play-fighting characteristics of agonistic behavior based on attack frequencies and repetitions within contact bouts.

To control for possible drug-induced locomotor deficits, hamsters were tested in a Lat maze following injections. Hamsters exhibit high levels of activity during the dark phase and, on average, traveled the equivalent of 60 meters during the 10-min test period. There was no effect of fluoxetine on locomotor activity in any groups except for adult hamsters given the high dose. This observation suggests that fluoxetine has nonspecific effects at high doses in adults. Following a similar pattern, contact time was inhibited in adults only at the high dose. Contact time was not affected by fluoxetine in juveniles. Furthermore, both the frequency of contact bouts and the number of attacks per bout were inhibited at the high dose of fluoxetine in adults. No such inhibition was seen in juveniles. These observations suggest that the effects of high doses of fluoxetine may not be specific to offensive responses in adults but may instead result from a general behavioral inhibition.

During puberty, there is a gradual transition in the targets of attacks performed by the resident (Taravosh-Lahn & Delville, 2004; Wommack et al., 2003). In juvenile hamsters (early puberty), the majority of attacks are directed at the face and cheeks of the intruder (play-fighting attacks), with a smaller percentage targeting the flanks (side attacks). In adults, the majority of attacks are directed at the lower belly and rump (adult attacks). At midpuberty, a substantial portion of attacks is directed at the flanks, whereas play-fighting attacks become rare and adult attacks start appearing. Treatment with a single dose of fluoxetine, both low and high, accelerated the maturation of attack targets. The percentage of side attacks was higher in fluoxetine-treated juveniles, whereas play-fighting attacks were reduced. In contrast, treatment with fluoxetine had no effect on attack targets in adult hamsters, as these hamsters only perform adult attacks. At least, treatment with fluoxetine in adults did not regress the maturation of the targets of attacks. Moreover, the effect observed in juveniles did not follow the same differential pattern of responsiveness observed with attack frequencies and attack repetitions. These data suggest that separate neural mechanisms underlie the control of attack frequency and attack repetition from the maturation of attack targets.

Previous studies on the maturation of attack targets focused on stress and cortisol in hamsters during puberty (Wommack, Salinas, & Delville, 2005; Wommack et al., 2003). Exposure to chronic social stress early in puberty accelerates the maturation of attack targets (Wommack et al., 2003). As puberty is marked by increasing release of cortisol in this species (Wommack et al., 2004, 2005), it was hypothesized that cortisol controls the maturation of attack types. As this hypothesis was confirmed (Wommack et al., 2005), it can now be argued that the effect of cortisol on the maturation of attack targets is mediated through a modulation of serotonin. It is noteworthy that although it took several days of treatment with a glucocorticosteroid to affect the maturation of attack targets (Wommack et al., 2005), the effect was replicated by a single dose of fluoxetine. The possible role of glucocorticosteroid on the maturation of serotonin systems is supported by the presence of corticosteroid receptors in the raphe nuclei (Cintra et al., 1994; Morimoto, Morita, Ozawa, Yokoyama, & Kawata, 1996) and the activation of tryptophan hydroxylase activity by corticosterone within the area (Azmitia & McEwen, 1974). Furthermore, as chronic social stress accelerates the maturation of attack targets, it also increases serotonin innervation of the AH (Delville et al.,

1998). Similar activation of serotonin systems has been reported in rats as stress exposure enhances serotonin release in various parts of the brain (Funada & Hara, 2001; Haller, Toth, & Halasz, 2005; Jordan, Kramer, Zukas, & Petty, 1994; Mendlin, Martin, Rueter, & Jacobs, 1996; Umriukhin, Wigger, Singewald, & Landgraf, 2002).

The density of serotonin-immunoreactive varicosities was analyzed for several areas involved in offensive responses and was compared between juveniles and adults. In two areas, the MePD and the AH, the density of serotonin-immunoreactive varicosities was elevated in adults. These data are interesting for several reasons. First, they confirm the possibility that serotonin availability increases in selective parts of the brain during puberty, correlating with the increasing responsiveness to high doses of fluoxetine during that period. Second, these data point to changes within the AH and MePD. The AH has been identified as a key area in the control of agonistic behavior in hamsters (Ferris et al., 1997, 1999; Jackson et al., 2005). Microinjections of serotonin receptor ligands within this area modulate offensive responses (Ferris et al., 1999). Activation of aggression in hamsters with pharmacological treatments correlates with increased density of serotonin innervation of the area (DeLeon, Grimes, Connor, & Melloni, 2002; Grimes & Melloni, 2002). It is possible that the maturation of agonistic behavior in hamsters is modulated by changes in serotonin within these areas. In addition, these data support the possibility that the same neural network controlling offensive responses in adults also controls offensive responses during play fighting in juveniles (Delville et al., 2003).

The differential effects of fluoxetine doses on attack frequency in juvenile hamsters can also be discussed in the context of serotonin receptors. To date, several subtypes of serotonin receptors have been involved in the control of aggression in adult hamsters. Activation of serotonin-1A receptors inhibits offensive aggression (Ferris et al., 1999; Joppa, Rowe, & Meisel, 1997; Knyshevski, Ricci, McCann, & Melloni, 2005). In contrast, activation of serotonin-3 receptors enhances aggression in hamsters (Ricci, Knyshevski, & Melloni, 2005). On the basis of these observations, it is possible that the differential effects on fluoxetine between juveniles and adults, and particularly the enhanced aggression after acute treatment with fluoxetine, is explained by an increase in the ratio of serotonin-1A to serotonin-3 receptors in the AH during puberty.

In summary, the present data partially support the hypothesis that differences in attack frequency between adult and juvenile hamsters are related to differences in serotonin availability. As juvenile hamsters are very active during play fighting, this behavior is associated with decreased serotonin availability and differential response to serotonin. In addition, our data also support the hypothesis that a single neural circuitry based on the AH controls agonistic behavior (such as play fighting and adult aggression) across puberty (Delville et al., 2003). The data presented in this study may also be relevant to occasional reports of undesirable effects (increased aggression or suicide) of serotonin reuptake inhibitors in adolescents (Constantino, Liberman, & Kincaid, 1997; Jick, Kaye, & Jick, 2004). It is possible that, in children as in hamsters, differential ratios of serotonin receptor subtypes across puberty may explain these detrimental effects of serotonin reuptake inhibitors.

References

Azmitia, E. C., Jr., & McEwen, B. S. (1974). Adrenalcortical influence on rat brain tryptophan hydroxylase activity. *Brain Research, 78,* 291–302.

Cervantes, M. C., David, J. T., Loyd, D. R., Salinas, J. A., & Delville, Y. (2005). Lead exposure alters the development of agonistic behavior in golden hamsters. *Developmental Psychobiology, 47,* 158–165.

Cervantes, M. C., Taravosh-Lahn, K., Wommack, J. C., & Delville, Y. (in press). Characterization of offensive responses during the maturation of play-fighting into aggression in male golden hamsters. *Developmental Psychobiology.*

Cintra, A., Zoli, M., Rosen, L., Agnati, L. F., Okret, S., Wikstrom, A. C., et al. (1994). Mapping and computer assisted morphometry and microdensitometry of glucocorticoid receptor immunoreactive neurons and glial cells in the rat central nervous system. *Neuroscience, 62,* 843–897.

Constantino, J. N., Liberman, M., & Kincaid, M. (1997). Effects of serotonin reuptake inhibitors on aggressive behavior in psychiatrically hospitalized adolescents: Results of an open trial. *Journal of Child and Adolescent Psychopharmacology, 7,* 31–44.

David, J. T., Cervantes, M. C., Trosky, K. A., Salinas, J. A., & Delville, Y. (2004). A neural network underlying individual differences in emotion and aggression in male golden hamsters. *Neuroscience, 126,* 567–578.

DeLeon, K. R., Grimes, J. M., Connor, D. F., & Melloni, R. H., Jr. (2002). Adolescent cocaine exposure and offensive aggression: Involvement of serotonin neural signaling and innervation in male Syrian hamsters. *Behavioural Brain Research, 133,* 211–220.

Delville, Y., David, J. T., Taravosh-Lahn, K., & Wommack, J. C. (2003). Stress and the development of agonistic behavior in golden hamsters. *Hormones and Behavior, 44,* 263–270.

Delville, Y., De Vries, G. J., & Ferris, C. F. (2000). Neural connections of the anterior hypothalamus and agonistic behavior in golden hamsters. *Brain, Behavior and Evolution, 55,* 53–76.

Delville, Y., Mansour, K. M., & Ferris, C. F. (1996a). Serotonin blocks vasopressin-facilitated offensive aggression: Interactions within the ventrolateral hypothalamus of golden hamsters. *Physiology & Behavior, 59,* 813–816.

Delville, Y., Mansour, K. M., & Ferris, C. F. (1996b). Testosterone facilitates aggression by modulating vasopressin receptors in the hypothalamus. *Physiology & Behavior, 60,* 25–29.

Delville, Y., Melloni, R. H., Jr., & Ferris, C. F. (1998). Behavioral and neurobiological consequences of social subjugation during puberty in golden hamsters. *Journal of Neuroscience, 18,* 2667–2672.

Delville, Y., Newman, M. L., Wommack, J. C., Taravosh-Lahn, K., & Cervantes, M. C. (2005). Development of aggression. In R. J. Nelson (Ed.), *Biology of aggression* (pp. 327–350). New York: Oxford University Press.

Ferris, C. F., Melloni, R. H., Jr., Koppel, G., Perry, K. W., Fuller, R. W., & Delville, Y. (1997). Vasopressin/serotonin interactions in the anterior hypothalamus control aggressive behavior in golden hamsters. *Journal of Neuroscience, 17,* 4331–4340.

Ferris, C. F., Stolberg, T., & Delville, Y. (1999). Serotonin regulation of aggressive behavior in male golden hamsters (*Mesocricetus auratus*). *Behavioral Neuroscience, 113,* 804–815.

Funada, M., & Hara, C. (2001). Differential effects of psychological stress on activation of the 5-hydroxytryptamine- and dopamine-containing neurons in the brain of freely moving rats. *Brain Research, 901,* 247–251.

Goldman, L., & Swanson, H. H. (1975). Developmental changes in preadult behavior in confined colonies of golden hamsters. *Developmental Psychobiology, 8,* 137–150.

Grimes, J. M., & Melloni, R. H., Jr. (2002). Serotonin modulates offensive attack in adolescent anabolic steroid-treated hamsters. *Pharmacology Biochemistry and Behavior, 73,* 713–721.

Haller, J., Toth, M., & Halasz, J. (2005). The activation of raphe seroto-

nergic neurons in normal and hypoarousal-driven aggression: A double labeling study in rats. *Behavioural Brain Research, 161,* 88–94.

Jackson, D., Burns, R., Trksak, G., Simeone, B., DeLeon, K. R., Connor, D. F., et al. (2005). Anterior hypothalamic vasopressin modulates the aggression-stimulating effects of adolescent cocaine exposure in Syrian hamsters. *Neuroscience, 133,* 635–646.

Jick, H., Kaye, J. A., & Jick, S. S. (2004). Antidepressants and the risk of suicidal behaviors. *Journal of the American Medical Association, 292,* 338–343.

Joppa, M. A., Rowe, R. K., & Meisel, R. L. (1997). Effects of serotonin 1A or 1B receptor agonists on social aggression in male and female Syrian hamsters. *Pharmacology Biochemistry and Behavior, 58,* 349–353.

Jordan, S., Kramer, G. L., Zukas, P. K., & Petty, F. (1994). Previous stress increases in vivo biogenic amine response to swim stress. *Neurochemical Research, 19,* 1521–1525.

Knyshevski, I., Ricci, L. A., McCann, T. E., & Melloni, R. H., Jr. (2005). Serotonin type-1A receptors modulate adolescent, cocaine-induced offensive aggression in hamsters. *Physiology & Behavior, 85,* 167–176.

Mendlin, A., Martin, F. J., Rueter, L. E., & Jacobs, B. L. (1996). Neuronal release of serotonin in the cerebellum of behaving rats: An in vivo microdialysis study. *Journal of Neurochemistry, 67,* 617–622.

Miller, L. L., Whitsett, J. M., Vandenbergh, J. G., & Colby, D. R. (1977). Physical and behavioral aspects of sexual maturation in male golden hamsters. *Journal of Comparative Physiological Psychology, 91,* 245–259.

Morimoto, M., Morita, N., Ozawa, H., Yokoyama, K., & Kawata, M. (1996). Distribution of glucocorticoid receptor immunoreactivity and mRNA in the rat brain: An immunohistochemical and in situ hybridization study. *Neuroscience Research, 26,* 235–269.

Pellis, S. M., & Pellis, V. C. (1988). Play-fighting in the Syrian golden hamster *Mesocricetus auratus Waterhouse,* and its relationship to serious fighting during postweaning development. *Developmental Psychobiology, 21,* 323–337.

Ricci, L. A., Knyshevski, I., & Melloni, R. H., Jr. (2005). Serotonin type 3 receptors stimulate offensive aggression in Syrian hamsters. *Behavioural Brain Research, 156,* 19–29.

Taravosh-Lahn, K., & Delville, Y. (2004). Aggressive behavior in female golden hamsters: Development and the effect of repeated social stress. *Hormones and Behavior, 46,* 428–435.

Umriukhin, A. E., Wigger, A., Singewald, N., & Landgraf, R. (2002). Hypothalamic and hippocampal release of serotonin in rats bred for hyper- or hypo-anxiety. *Stress, 5,* 299–305.

Vomachka, A. J., & Greenwald, G. S. (1979). The development of gonadotropin and steroid hormone patterns in male and female hamsters from birth to puberty. *Endocrinology, 105,* 960–966.

Weinert, D., Fritzsche, P., & Gattermann, R. (2001). Activity rhythms of wild and laboratory golden hamsters (*Mesocricetus auratus*) under entrained and free-running conditions. *Chronobiology International, 18,* 921–932.

Wommack, J. C., Salinas, A., & Delville, Y. (2005). Glucocorticoids and the development of agonistic behaviour during puberty in male golden hamsters. *Journal of Neuroendocrinology, 17,* 781–787.

Wommack, J. C., Salinas, A., Melloni, R. H., Jr., & Delville, Y. (2004). Behavioural and neuroendocrine adaptations to repeated stress during puberty in male golden hamsters. *Journal of Neuroendocrinology, 16,* 767–775.

Wommack, J. C., Taravosh-Lahn, K., David, J. T., & Delville, Y. (2003). Repeated exposure to social stress alters the development of agonistic behavior in male golden hamsters. *Hormones and Behavior, 43,* 229–236.

Received April 5, 2006
Revision received June 26, 2006
Accepted July 5, 2006 ■