Altered Profiles of Spontaneous Novelty Seeking, Impulsive Behavior, and Response to D-Amphetamine in Rats Perinatally Exposed to Bisphenol A

Walter Adriani,[1] Daniele Della Seta,[2] Francesco Dessì-Fulgheri,[3] Francesca Farabollini,[2] and Giovanni Laviola[1]

[1]Section of Behavioral Pathophysiology, Laboratorio Fisiopatologia O.S., Istituto Superiore di Sanità, Roma, Italy; [2]Institute of Human Physiology, University of Siena, Siena, Italy; [3]Department of Animal Biology and Genetics, University of Florence, Florence, Italy

Bisphenol A (BPA) is an environmental estrogen with potentially averse effects on public health. We studied the long-term effects of perinatal exposure to BPA on later behavior in adult rats of both sexes. BPA or vehicle was administered orally to mother rats from mating to pups' weaning, at a concentration (0.040 mg/kg) within the range of human exposure. The offspring of both sexes were tested at adolescence (postnatal days 35–45) for novelty preference (experiment 1). After a 3-day familiarization to one side of a two-chamber apparatus, on day 4 rats were allowed to freely explore the whole apparatus. BPA-exposed females spent significantly less time than did controls in exploration of the novel side (i.e., increased neophobia), whereas no effect was found in the male group. At adulthood, the same animals were food deprived and tested for profiles of impulsive behavior (experiment 2), in operant chambers provided with two nose-poking holes (delivering either five or one food pellet). After the establishment of a baseline preference for the large reinforcer, a delay was introduced before the delivery of the five food pellets, which was progressively increased each day (10, 20, 30, 45, 60, 80, 100 sec). As expected, all animals exhibited a progressive shift toward the immediate but smaller reinforcer. A reduced level of impulsive behavior (i.e., a shift to the right in the intolerance–delay curve) was evidenced in BPA-treated rats. The frequency of inadequate responding (during the length of the delay) also provided a measure of restless behavior. Interestingly, the profile of BPA-treated males was feminized, strongly resembling that of control females. Animals were then tested (experiment 3) for the response to an amphetamine challenge (1 mg/kg, subcutaneously). The drug-induced increment activity was significantly less marked in BPA-treated male rats compared with controls. These findings provide clear indirect evidence of long-term alterations in brain monoaminergic function after perinatal BPA exposure. This may be a cause for concern for public health, confirming that exposure to a weak environmental estrogen in the period of sexual differentiation of the brain can influence adult behavior. *Key words:* **amphetamine, behavior, bisphenol A, environment, impulsivity, novelty-seeking, pollutants, rats.** *Environ Health Perspect* **111:395–401 (2003). doi:10.1289/ehp.5856 available via** *http://dx.doi.org/* **[Online 30 October 2002]**

There is increasing concern about the negative impact on public health of environmental chemicals with estrogenic activity (Carlsen et al. 1992; Guillette et al. 1996; Wolff et al. 1993). The ability of estrogenic hormones to affect sexual differentiation of the brain during a critical period of perinatal life is well known (Arnold and Gorski 1984; Hutchison 1997). In addition to reproductive and sexual behavior, a variety of behavioral patterns are organized and sexually differentiated in rodents under the influence of perinatal gonadal hormones (Beatty 1979; McClusky 1981; McEwen 1992). Recent advances in the neurosciences have shown that estrogens interact with the dopaminergic (Alderson and Baum 1981; Becker 1999; Euvrard et al. 1980; Hruska and Pitman 1982; Menniti and Baum 1981; Peris et al. 1991) and the serotonergic (Osterlund et al. 2000; Osterlund and Hurd 1998) brain systems. Perinatal exposure to estrogenic pollutants could hence alter development of these major neurochemical pathways (see, e.g., Christian and Gillies 1999; Lilienthal et al. 1997), leading to permanent neurobehavioral alterations in the offspring.

Bisphenol A (BPA) is a particularly important environmental estrogen. It is not only widespread but also potentially ingested by humans, being released by polycarbonate plastics, the lining of food cans, and dental sealants (Brotons et al. 1995; Olea et al. 1996). Prenatal exposure to BPA can affect the development and function of reproductive organs as well as adult sexual behavior, especially in male rodents and in their offspring (Atanassova et al. 2000; Dessì-Fulgheri et al. 2002; Farabollini et al. 2002; Fisher et al. 1999; Howdeshell et al. 1999; Rubin et al. 2001; Vom Saal et al. 1995, 1998; Williams et al. 2001a, 2001b). Perinatal exposure to BPA has also been implicated in altered profiles of nonsocial behaviors, resulting in a reduced motivation to explore and a reduced anxiety in the male offspring (Farabollini et al. 1999). In the present work, we wanted to extend the analysis of the consequences of early BPA exposure on nonsexual behaviors, investigating behaviors that rely upon central serotonergic and dopaminergic brain systems. Specifically, the serotonergic system is thought to be important for impulse control across a wide range of behaviors (Soubrié 1986), whereas the dopaminergic system is strongly involved in mediating motivation and reward (Robbins and Everitt 1996; Wise 1996).

The dopaminergic system is particularly important for the expression of novelty-seeking behavior (Bardo et al. 1996; Pierce et al. 1990). Both humans and animals have a natural need to search for novel and rewarding stimuli (Renner 1990; Zuckerman 1994), and the experience of novelty is rewarding via the activation of the mesolimbic dopaminergic system (Rebec et al. 1997a, 1997b). This behavioral trait is particularly expressed during adolescence in both humans (Arnett 1992; Zuckerman 1994) and animal models (Adriani et al. 1998; Bardo et al. 1996; Macrì et al. 2002). In rodents, adolescence is classically defined as the ontogenetic period including the week preceding the onset of puberty and the first few days thereafter (Spear and Brake 1983). We tested the hypothesis that possible alterations in coping with novelty, deriving from perinatal exposure to an estrogenic pollutant, could be easily detectable around puberty. The latter is indeed characterized by the onset of prominent hormonal regulation. For this reason, BPA-exposed rats of both sexes were assessed in a novelty preference test (Bardo et al. 1988; Misslin and Ropartz 1981) during adolescence (Spear and Brake 1983).

The role of the serotonergic system in modulating premature and impulsive responding is widely recognized on both clinical (Linnoila et al. 1983) and preclinical literature (Soubrié 1986). Impulsivity can be defined in several ways, including *a*) the "failure to resist

Address correspondence to G. Laviola, Section of Behavioral Pathophysiology, Lab. Fisiopatologia O.S., Istituto Superiore di Sanità, viale Regina Elena 299, I-00161 Roma, Italy. Telephone: 39-06-4990-2105. Fax: 39-06-495-7821. E-mail: laviola@iss.it

We thank M. Sbragi, who developed the computer software for the operant chambers, and M. Rea for her valuable collaboration in the third experiment (behavioral observation and data analysis).

This research was supported as part of the intramural grant to G.L., Research Project on "Psychobiological risk or protection factors for behavioral disorders and vulnerability to recreational substances abuse during development," Nervous and Mental Disorders Research Area, Istituto Superiore di Sanità, Italy, and by a grant to F.D.-F., Research Project COFIN titled "Behavior as a biomarker of the effects of oestrogenic pollutants in higher vertebrates," from MURST, Italy. A "Giuseppe LEVI" bursary from the Accademia Nazionale dei Lincei (Italy) supported W.A.

Received 25 June 2002; accepted 20 September 2002.

an impulse, drive, or temptation" (Evenden 1999); *b*) responding without consideration of alternatives and/or consequences; or *c*) behaving in a way that is adequate to the environmental contingency. Many different aspects of impulsivity have been studied with operant paradigms in laboratory settings (Bradshaw and Szabadi 1992; Evenden 1999; Richards et al. 1997, 1999). One of the most widely adopted paradigms assumes that impulsive subjects are intolerant to situations when reward is delayed. Smaller immediate reinforcers are preferred to larger rewards, which come only after a delay (Bizot et al. 1999; Evenden and Ryan 1996, 1999; Logue 1988; Thiebot et al. 1985). In the present study, we evaluated the possibility that perinatal exposure to BPA may influence the development of the serotonergic system in adult animals through the impulsivity test.

The specificity of the developmental changes affecting a central neurochemical system can be evaluated by assessing the effects of a psychoactive agent targeting that system upon the behavioral responses known to be modulated by that system. For this reason, it seemed appropriate to evaluate the increase of locomotion and rearing behavior that follow amphetamine administration (Kelly et al. 1975), because it is well known that release of dopamine within the dorsal and ventral striatum is involved in such a behavioral change (Staton and Solomon 1984). For this study, we considered a potential alteration in the behavioral effects of amphetamine administration an index of BPA-induced long-term effects on the function of the brain dopaminergic system.

We studied the effects of precocious exposure to BPA [at concentrations within the range of human exposure and not teratogenic (Brotons et al. 1995; Olea et al. 1996)]. To this purpose, we administered BPA orally to pregnant females from mating to weaning day. The offspring were then tested for novelty preference during adolescence. When adult, the same subjects were also tested for intolerance to delay and for amphetamine-induced behaviors in an open field.

Methods

Subjects, breeding, and rearing conditions. Sprague-Dawley rats were housed in an air-conditioned room (temperature, 21 ± 1°C; relative humidity, 60 ± 10%), with a 12-hr light/dark cycle (lights off from 2100 to 0900 hr). Water and food (Enriched Standard Diet; Mucedola, Settimo Milanese, Italy) were available *ad libitum*. Breeding pairs were formed and housed in Plexiglas cages, with metal tops and sawdust bedding. After detection of the vaginal plug, the male was removed, and the females were housed individually. The day of delivery was considered postnatal day (PND) 0; pups were weaned on PND 25 and housed in groups of three, according to sex. One male and one female per litter were observed in the present study. Animals were tested during adolescence (PND 30–45) for novelty seeking and when adult (PNDs > 70) for impulsivity and response to amphetamine.

The estrogenic pollutant BPA (Fluka Chemie Ag, Buchs, Switzerland) was administered daily to females (n = 9) from mating day to weaning day. The substance was dissolved in arachis oil at a concentration of 0.04 mg/kg, which was administered orally by micropipette, the volume administered depending on body weight. Control females (n = 9) received arachis oil without BPA. Because animals were trained to receive the oil before mating, this procedure was not stressful.

Experiment 1: novelty preference test. Animals of both sexes were tested for levels of novelty seeking during adolescence. The experimental apparatus consisted of an opaque Plexiglas box with smooth walls (70 × 30 × 35 cm), subdivided into two compartments. The connecting door between the two compartments could be closed by means of a temporary partition. One compartment had a wide-mesh floor, whereas the other had narrow mesh. Animals were video recorded and later scored for measures of time spent in each compartment and activity rate in each compartment. To evaluate the activity rate, the floor of each compartment was subdivided into three sections by lines placed on the video screen at the time of video-recording analysis, and the number of line crossings (with both forepaws) was scored.

The whole experimental schedule took 5 days, each subject from both age groups being tested between 1000 and 1800 hr. Testing of different experimental groups was counterbalanced across time. The test was carried out under dim illumination. The floor of the apparatus was cleaned after each animal was tested. During the familiarization phase (days 1–3), animals were gently placed for 20 min in one compartment of the apparatus. During the novelty preference test (on day 4), animals were placed in the familiar compartment for a 5-min session. The partition separating the two compartments of the apparatus was then removed, and rats were thus allowed to freely explore the whole apparatus (both the familiar and the novel sides) for 24 min.

Experiment 2: impulsivity test. When adult, the same animals were tested for levels of impulsivity. Before the schedule started, animals were food deprived (80% of free-feeding weight; see Table 1) to increase their motivation to work for food delivery. Each animal was then placed daily in a computer-controlled operant chamber (Coulbourn Instruments, Allentown, PA, USA), provided with two nose-poking holes, a chamber light, a feeder device, a magazine where pellets (45 mg; BioServ, Frenchtown, NJ, USA) were dropped, and a magazine light. The nose poking in either hole was detected by a photocell and was recorded by a computer, which also controlled food delivery. After a 30-min session, animals were returned to their home cages, where they were given standard chow (~8 g each), to keep animals at 80–85% of their free-feeding weight.

During the training phase (1 week), nose poking in one of the two holes [called the "immediate and small" (IAS) hole] resulted in the delivery of one pellet of food, whereas nose poking in the other hole ["large and delayed" (LAD) hole] resulted in the delivery of five pellets of food. After nose poking and before food delivery, the chamber light was turned on for 1 sec. After the food delivery, the magazine light was turned on for 25 sec, during which additional nose poking was recorded but was without any scheduled consequence (time out).

During the testing phase (1 week), a delay was inserted between nose poking in the LAD hole and the delivery of the five pellets. The chamber light was turned on during the length of this delay. Any additional nose poking taking place during this time interval was recorded but was without any consequence ["inadequate responding" (Sagvolden and Sergeant 1998; Sagvolden 2000)]. The delay was kept fixed for each daily session and was increased progressively over subsequent days (0, 10, 20, 40, 60, 80, 100 sec). The dependent variables were the percentage of choice between the LAD and IAS holes and the frequency of inadequate nose poking.

Experiment 3: open field with amphetamine. One week after the impulsivity test, all animals were tested for response to amphetamine in an open-field apparatus. This consisted of an opaque Plexiglas rectangular box with smooth gray walls and floor (70 × 30 × 35 cm). D-Amphetamine (AMPH; 1 mg/kg) was dissolved in saline (SAL; NaCl 0.9%) and injected subcutaneously in a volume of 1 mL/kg body weight. Approximately 15 min after the injection with either SAL or AMPH,

Table 1. Mean (± SE) body weight before food deprivation (day 0) and during the schedule (days 3–12) in experiment 3.

	Males		Females	
Day	Control	BPA-treated	Control	BPA-treated
0	478 ± 11	482 ± 1	284 ± 10	302 ± 9
3	441 ± 9	449 ± 11	265 ± 7	278 ± 8
6	410 ± 9	432 ± 10	258 ± 8	269 ± 9
9	410 ± 8	414 ± 11	251 ± 6	262 ± 8
12	383 ± 9	389 ± 11	235 ± 6	246 ± 8

animals were placed in the open field for a single 30-min session. The behavioral profile expressed by each animal was video recorded and later scored by a treatment-blinded individual, using a computer and specific software (The Observer, version 2.0 for DOS; Noldus Information Technology, Wageningen, The Netherlands). This allowed a detailed analysis of several parameters, including latency, frequency, and duration of each behavior. Three behaviors were scored: rearing (body in vertical position), grooming (mouth or paws on body), and crossing (the floor of each compartment was subdivided into three sections by lines placed on the video screen at the time of video-recording analysis, and the number of line crossings with both forepaws was scored).

Design and data analysis. Data were analyzed by multifactorial analysis of variance (ANOVA). The general design of all experiments was two sex (male vs. female) × two treatment (BPA vs. oil) × subject. For the novelty-seeking paradigm (experiment 1), a side (familiar vs. novel) and a time factor were added. For the impulsivity paradigm (experiment 2), a delay factor (0, 10, 20, 40, 60, 80, 100 sec) was added. In the open-field test (experiment 3), a drug factor (SAL vs. AMPH) was added. Multiple comparisons within significant interactions were performed with the Tukey HSD test.

Results

Experiment 1: novelty preference test. **Activity rate.** The four-way ANOVA yielded significance for the time effect [$F(5,160) = 4.96$, $p < 0.001$] and for the sex by time interaction [$F(5,160) = 2.49$, $p < 0.05$], indicating that the time-course profile of activity during the test was markedly different in the two sexes. On this basis, and to analyze more specifically the effects of BPA exposure, the two sexes were analyzed separately by a three-way ANOVA.

For males (Figure 1C), the ANOVA yielded significance for the time by side interaction [$F(2,32) = 3.99$, $p < 0.05$]. Specifically, when animals were in the novel compartment, the activity rate was particularly elevated in the first part of the session, decreasing thereafter. Conversely, in the familiar compartment, the activity profile was flat during the whole session (data not shown). Moreover, a treatment × side × time interaction [$F(2,32) = 9.28$, $p < 0.05$] emerged. The prenatal exposure to BPA resulted in higher activity levels than for controls in the novel environment, especially at the end of the session. In other words, the habituation profile was less pronounced in BPA-exposed rats. Conversely, levels of activity in the familiar were not affected (data not shown).

For females (Figure 1D) the ANOVA yielded significance for the side by treatment interaction [$F(1,16) = 10.53$, $p < 0.01$]. As in males, the perinatal exposure to BPA resulted in higher levels of activity than for controls in the novel environment. Conversely, levels of activity in the familiar compartment were not affected (data not shown).

Novelty preference. In the three-way ANOVA, the main effect of time was significant [$F(5,160) = 12.26$, $p < 0.01$]. The novelty preference increased as session progressed. The ANOVA yielded significance for the sex by treatment interaction [$F(1,32) = 4.40$, $p < 0.05$]. As a whole, early exposure to BPA produced a marked reduction of time spent in the novel environment in females (Figure 1B), whereas the group of males was not affected (Figure 1A).

Separate analyses confirmed this picture. For females, but not males, the two-way ANOVA yielded significance for the main effect of treatment [$F(1,16) = 10.44$, $p < 0.01$]. Multiple comparisons performed within the female group revealed that, as a consequence of perinatal BPA exposure, a reduction of time spent in the novel environment was found at the beginning and at the end of the session.

Experiment 2: impulsivity test. **Choice between reinforcers.** As expected, after the training period, animals of both sexes developed a significant preference for the LAD hole, delivering the large reinforcer (Figure 2). The preference also progressively shifted toward the hole delivering the immediate reinforcer as the length of the delay was increased [delay, $F(6,192) = 31.6$, $p < 0.01$], but no evidence of a sex difference was found in the ANOVA. Interestingly, a main effect of treatment [$F(1,32) = 4.28$, $p < 0.05$] revealed that adult rats exposed perinatally to BPA were associated with a more marked preference for the LAD reinforcer during the whole experiment. As a whole, this profile suggests a shift to the right in the delay–response curve (i.e., reduced impulsivity) in rats of both sexes.

Inadequate responding. Because the nose poking in either hole during the course of the delay had no scheduled consequences, it was considered an "inadequate response" (Sagvolden 2000; Sagvolden and Sergeant 1998). Such a measure provides an index of inability to inhibit an unnecessary response. As the length of the delay was increased, the inadequate nose poking in the LAD hole was progressively reduced, whereas the inadequate nose poking in the IAS hole increased progressively

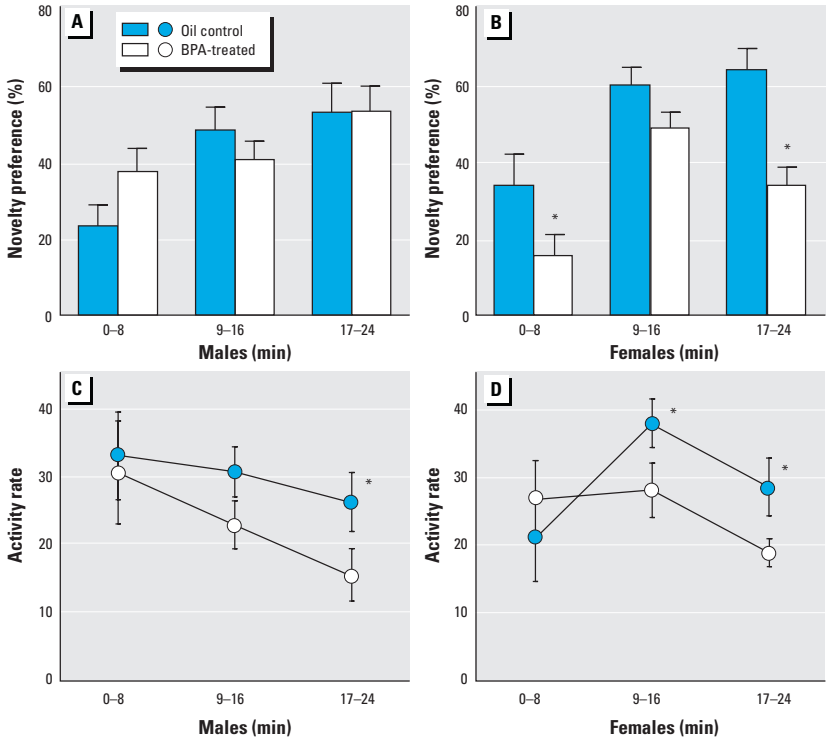

Figure 1. (*A,B*) Mean (± SE) percentage of time spent in the novel compartment by subjects of both sexes on testing day (experiment 1). (*C,D*) Mean (± SE) activity rate, measured as number of line crossings per minute, shown by subjects of both sexes in the novel compartment on testing day. During the pretreatment period (days 1–3), subjects were familiarized to one compartment. On testing day, animals were placed in the familiar compartment. After 5 min, a partition was removed and subjects were allowed free access to a novel compartment of the apparatus for a 24-min session.
*$p < 0.05$ in comparisons between BPA and control perinatal treatments ($n = 9$).

[delay, $F(6,192) = 14.9$, $p < 0.01$; delay × hole, $F(6,192) = 49.1$, $p < 0.01$]. Such a finding suggests that, during the length of the delay, when they had to wait for the large reinforcer, rats were demanding more and more the small and immediate one. Interestingly, a significant main effect of sex [$F(1,32) = 4.44$, $p < 0.05$] and significant sex by treatment interaction [$F(1,32) = 4.25$, $p < 0.05$] were found. To better depict the profile, data from the two sexes were analyzed separately.

For males, a main effect of treatment [$F(1,16) = 8.29$, $p < 0.05$] as well as a delay by treatment interaction [$F(6,96) = 2.12$, $p < 0.05$] emerged. Multiple comparisons revealed that, as the delay increased, adult rats were associated with elevated nose poking. Interestingly, a significant delay by hole by treatment interaction [$F(6,96) = 3.24$, $p < 0.01$] appeared. Multiple comparisons revealed that, when the length of the delay was set to 1 min or more, BPA-exposed rats were specifically associated with a significantly lower frequency of nose poking in the IAS hole (Figure 3A). It is interesting to note that all these interactions were not significant within the female group; that is, female subjects were apparently not affected by BPA exposure (Figure 3B). Furthermore, early BPA exposure results in males whose profile is comparable with that expressed by females.

Experiment 3: Open field with amphetamine. **Crossing.** The ANOVA yielded a main effect of drug [$F(1,28) = 51.3$, $p < 0.01$], with AMPH injection resulting in elevation of crossing frequency. Interestingly, a main effect of sex [$F(1,28) = 6.64$, $p < 0.05$] and a sex by treatment interaction [$F(1,28) = 9.49$, $p < 0.01$] also appeared.

To better depict the effects, we analyzed the data from the two sexes separately (see Figure 4C,D). For males, the ANOVA evidenced a main effect of drug [$F(1,14) = 37.7$, $p < 0.01$], with AMPH resulting in elevation of crossing frequency. Moreover, a main effect of treatment [$F(1,14) = 10.7$, $p < 0.01$] and a drug by treatment interaction [$F(1,14) = 6.34$, $p < 0.05$] emerged. Specifically, multiple comparisons revealed that AMPH administration resulted in elevation of crossing in control but not in treated subjects. Conversely, for females, only a main effect of drug [$F(1,14) = 20.5$, $p < 0.01$] appeared. AMPH administration resulted in elevation of crossing in both control and BPA-treated subjects. As a whole, these results suggest that BPA exposure impaired the response to AMPH only in male subjects.

Rearing. The ANOVA yielded a main effect of drug [$F(1,28) = 22.5$, $p < 0.01$], AMPH resulting in elevation of rearing. Interestingly, a main effect of sex just missed significance [$F(1,28) = 3.27$, $p < 0.081$] and the sex by treatment interaction was significant [$F(1,28) = 6.25$, $p < 0.05$]. To better depict this effect, the two sexes were analyzed separately (Figure 4A,B). For males, the ANOVA evidenced a main effect of drug [$F(1,14) = 18.5$, $p < 0.01$] and treatment [$F(1,14) = 7.03$, $p < 0.05$]. Specifically, as is evident from Figure 4A, AMPH-induced elevation of rearing was less marked in BPA-treated than in control subjects. Conversely, for females, only a main effect of drug [$F(1,14) = 8.19$, $p < 0.05$] appeared. Specifically, AMPH administration resulted in elevation of rearing in both control and BPA-treated subjects. As a whole, these results suggest that BPA exposure impaired the response to AMPH in male subjects.

Discussion

As a whole, the present results can be summarized as follows: *a*) Rats of both sexes, perinatally exposed to BPA and tested during adolescence for novelty seeking, were associated with more marked levels of novelty-induced hyperactivity, compared with controls. However, BPA-exposed females spent a lower percentage of time in the novel environment (an index of neophobia). *b*) BPA-exposed rats of both sexes were associated with a more marked preference for the LAD reinforcer during the whole experiment (an index of decreased impulsivity). Compared with controls, BPA-exposed males exhibited a feminization in the frequency of inadequate nose poking at the IAS hole during the length of the delay. *c*) As expected, AMPH injection induced an elevation of crossing and rearing in control male subjects and in both groups of females. Perinatal BPA exposure was able to impair the classical response to AMPH in male subjects.

Novelty seeking in adolescent rats. Periadolescent rats and mice express elevated levels of behavioral activation in specific forms. For instance, they show elevated levels of social play and affiliative behaviors (Meaney and Stewart 1981; Panksepp 1981) that progressively shift toward aggressive and competitive behaviors (Terranova et al. 1993, 1998). Moreover, rodents at this age exhibit a marked peak in novelty-seeking behavior (Adriani et al. 1998; Bardo et al. 1996) and low levels of exploration-induced anxiety (Macrì et al. 2002). The psychobiology of novelty-seeking behavior has been studied in mice and rats. Specifically, the dopaminergic system has been widely implicated in mediating the

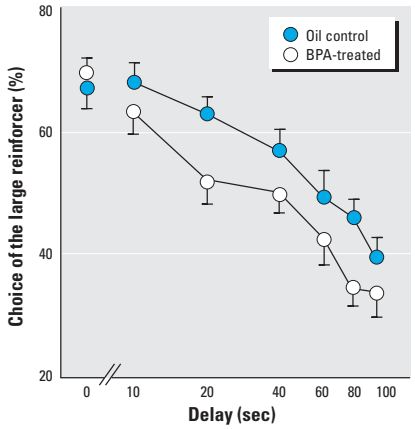

Figure 2. Mean (± SE) choice (%) of the large reinforcer, demanded by nose poking at the LAD hole, shown by rats during the test for impulsivity (experiment 2). These data reveal that, as the length of the delay increased, animals increased demanding the small but immediate reinforcement and decreased demanding the larger but delayed one. A shift to the right of the whole curve (i.e., a profile of reduced impulsivity) was evident in BPA-exposed rats compared with controls. In the absence of significant differences, data from the two sexes were collapsed ($n = 18$).

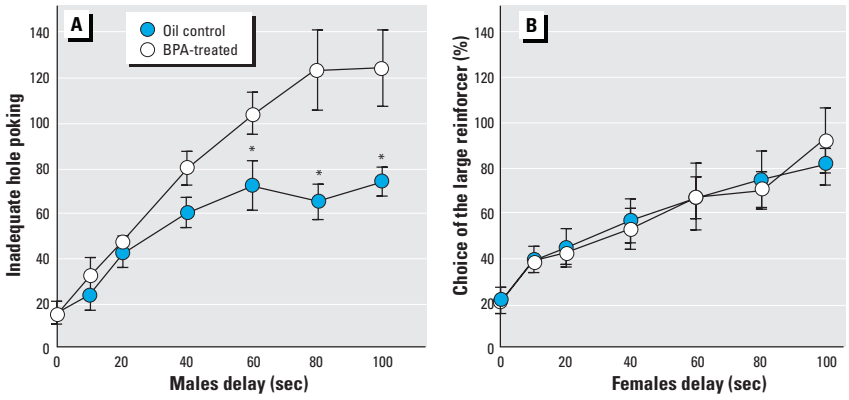

Figure 3. Mean (± SE) frequency of inadequate responding at the IAS hole (i.e., nose poking during the length of the delay, when it was without any consequence) shown by rats during the test for impulsivity (experiment 2). These data reveal that, when animals were waiting for the delivery of the large reinforcer, they failed to rest and were demanding the immediate one. A clear-cut demasculinization in the restlessness profile was evident.
*$p < 0.05$ in multiple comparisons between BPA and control perinatal treatments ($n = 9$).

incentive response to novelty (Bardo et al. 1996), whereas the limbic–hypothalamo–pituitary–adrenal axis determines the individual stressful responses to novelty (Kabbaj et al. 2000).

In the present experiment, female rats spent less time than did males in the new environment at the beginning of the free-choice exploration, suggesting a lower interest of females in exploring the novel side. These findings are consistent with other previous results, suggesting that females show lower levels of novelty seeking than do males in both rats (Hughes 1968) and mice (Palanza et al. 2001). Compared with controls, BPA-treated females spent a minor percentage of time in the novel compartment of the apparatus, remaining most of the time in the familiar compartment. These data indicate that, rather than being attractive as is normally reported for rats (Bardo et al. 1988), the experience of novelty was avoided by females after maternal BPA exposure. In other words, prenatal exposure to BPA was apparently responsible for an increased neophobia in adolescent female rats. In a previous study, parameters of motor activity and motivation to explore were depressed in adult female rats after maternal exposure to BPA (Farabollini et al. 1999). These findings suggest that, compared with control subjects, BPA-treated females were less prone to explore a novel environment.

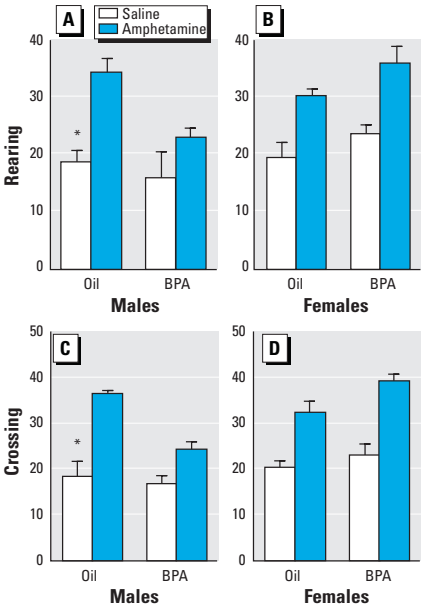

Figure 4. Mean (± SE) frequency of rearing (*A,B*) and crossing (*C,D*), shown by rats of both sexes in the open-field test (experiment 3). Subjects received either a SAL ($n = 4$) or an AMPH (1 mg/kg, $n = 5$) injection immediately before being placed for 30 min in the open field.

*$p < 0.05$ in multiple comparisons performed between SAL- and AMPH-injected subjects.

Regarding locomotion in the novel compartment, rats treated with BPA expressed elevated levels of activity and a less marked profile of habituation, an effect that was evident in both sexes. In the novelty-seeking test, exploratory activity and emotional reactivity represent two different dimensions based on different mechanisms (Zimmermann et al. 2001). A profile of hyperlocomotion during exploration of novel environments has been proposed as an index of novelty-induced stress (Exner and Clark 1993; Misslin and Ropartz 1981; Misslin et al. 1982). Present data may suggest that prenatal treatment with BPA produced rats that were more likely to experience novelty-induced stress during adolescence or, alternatively, a slowing down of the process of habituation. This behavioral profile could be related to alterations in the function of brain neurochemical systems involved in the locomotor response to novelty-induced stress and/or in the locomotor habituation to novelty.

Impulsive behavior. Impulsivity, defined as a reduced ability to tolerate a delay of gratification (Evenden 1999), has been studied in rats by means of various procedures, providing a choice between a large but delayed food reinforcement versus a smaller and immediate one (Bizot et al. 1999; Evenden and Ryan 1996, 1999). Delay has actually been shown to have a discounting effect on the subjective value of a given reinforcement (Bradshaw and Szabadi 1992; Richards et al. 1997).

In the present study, food-restricted animals were trained in operant chambers, where nose poking resulted in food delivery. As expected, all animals significantly preferred the hole associated with the large reinforcement (LAD hole) and also exhibited a shift toward the small, immediate reinforcement (IAS hole) as the length of the delay was increased. Rats exposed perinatally to BPA were associated with a more marked preference for the LAD reinforcer during the whole experiment—that is, with a rightward shift of the delay–preference curve—suggesting a reduction of impulsive behavior. In previous studies involving a similar paradigm, a marked increment in the preference for the large-but-delayed reward was induced by serotonin uptake inhibitors such as indalpine, zimelidine (Thiebot et al. 1985), fluoxetine, and fluvoxamine (Bizot et al. 1999). These data support the idea that serotonergic mechanisms are involved in the regulation of impulsive behavior, suggesting that an elevated serotonergic tone may result in elevated tolerance to reward delays. On this basis, it may be supposed that perinatal BPA exposure affected the ontogenesis of this central neurochemical system.

Nose poking in either hole during the length of the delay had no scheduled consequences. However, in the course of the present experiment, animals kept on demanding the food reinforcement even during the signaled nonreinforced component of the schedule. This might happen because animals were unable to modify response patterns with changes in the experimental contingency, being under the behavioral urge of doing something and unable to simply wait. This kind of behavior has been defined as "inadequate responding" (Sagvolden 2000; Sagvolden and Sergeant 1998; Sagvolden et al. 1998), and its measure might hence provide an index of restlessness and reduced ability to wait.

Interestingly, a sexual difference emerged, both at basal level and in the response to BPA: *a*) Females showed lower levels of inadequate responding than did the corresponding group of males; *b*) no effect of BPA exposure was found in females; and *c*) levels shown by BPA-exposed males resembled those shown by both groups of females. Results in control subjects suggest that males have a stronger preference for the immediate reinforcer than do females, which is progressively more expressed during the length of the delay. Alternatively, these results suggest that male subjects are less able than females to inhibit nose poking behavior during the delay. Interestingly, BPA-exposed males were specifically associated with lower levels of inadequate nose poking in the inactive hole, compared with controls. This suggests that BPA-treated males are less restless and more tolerant to the delay and/or more able to inhibit the inadequate behavior. Interestingly, early BPA exposure results in males whose profile is comparable with that expressed by females, suggesting a demasculinization for this measure. Consistently, modifications of sociosexual behavior in the direction of a demasculinization have been observed in adult male rats perinatally exposed to BPA (Farabollini et al. 2002).

Open-field test and response to amphetamine. As expected, the AMPH-induced elevation of both crossing and rearing was significantly reduced in BPA-treated male subjects. Such a picture suggests that early BPA exposure impaired the function of central neurochemical systems targeted by AMPH in the male offspring. A reduced dopaminergic function can be hypothesized for BPA-exposed males, which may also partially account for the particular hypoactivity shown by these subjects during the length of the delay in experiment 2 (discussed above). We may suppose that perinatal BPA exposure interacted with some steps in the development and organization of the dopaminergic system during the perinatal period of male offspring.

Regarding possible mechanisms, BPA exhibits weak estrogenic activity in adult rats of both sexes. Specifically, BPA administration causes a significant increase in uterus and vagina weights in ovariectomized females (Kim et al. 2001), whereas it directly inhibits testicular functions and produces a reduction in the negative feedback of testosterone (Tohei et al. 2001). Long-term exposure of adult female rats to BPA induces modifications in β-estrogen receptor immunoreactivity in various brain areas regulating reproductive and maternal behavior (Aloisi et al. 2001). Many studies have addressed the adverse effects of perinatal exposure to BPA on various indexes of sexual development and maturation (Atanassova et al. 2000; Fisher et al. 1999; Rubin et al. 2001; Williams et al. 2001a, 2001b). Unfortunately, little is known about the effect of estrogen-like compounds on developing monoamine systems. One paper reported, however, that intrauterine exposure to estradiol has a significant effect on the organization of monoamine systems within the fetal hypothalamus (Kaylor et al. 1984). More is known about interactions of estrogens with the adult dopaminergic and serotonergic systems (for a review, see, e.g., Cyr et al. 2002; Dluzen 2000; Fink et al. 1996, 1999; Rubinow et al. 1998).

Conclusions

As a whole, perinatal treatment with the estrogenic pollutant BPA resulted in marked alterations in rats' behavioral repertoire. Specifically, an increased novelty-induced stress and/or a reduced habituation to novelty was found during adolescence, as well as reduced levels of impulsivity at adulthood. Both findings may well be seen as indexes of a reduced reactivity or readiness to react to environmental challenges. It could be argued that BPA exposure resulted in individuals that do not easily adapt to environmental changes (see Benus et al. 1987).

Interestingly, some of these effects were sex dependent. The perinatal treatment with BPA affected the restlessness profile in male rats, with BPA-treated animals becoming undistinguishable from females. This finding, together with the reduced sensitivity of BPA-treated adult males to AMPH, suggests that the perinatal exposure to BPA interacts with some steps in the organization of the serotonergic and dopaminergic neural systems in the male offspring. On the contrary, perinatal BPA exposure produced neophobia in adolescent females but not in males. This effect was possibly determined via a different mechanism from that controlling impulsivity, because BPA exposure had no effect upon behavior of adult females in the impulsivity test or in the open-field test. On the basis of the scientific literature, the various behavioral alterations reported in the present study could be ascribed to an altered development of dopaminergic and/or serotonergic pathways. It can be hypothesized that both these systems were affected by perinatal BPA treatment, but further work is needed to clarify the neural basis of long-term neurobehavioral deficits induced by BPA.

The present results acquire even more importance on the basis of recent reports, indicating that performance in operant tasks is used also in children to evaluate adverse consequences of exposure to polychlorinated biphenyls (Stewart et al. 2001). As a general conclusion, the present findings provide indirect evidence of long-term consequences of perinatal BPA exposure at the level of neurobehavioral development. These alterations should be further investigated by means of biochemical testing. However, our results might be a cause of concern for public health, indicating that exposure to a weak environmental estrogen in the period of sexual differentiation of the brain may influence adult behavior. Further research is needed to better understand which exposure levels would not be potentially dangerous for human health.

References

Adriani W, Chiarotti F, Laviola G. 1998. Elevated novelty seeking and peculiar D-amphetamine sensitization in periadolescent mice. Behav Neurosci 112:1152–1166.

Alderson LM, Baum MJ. 1981. Differential effects of gonadal steroids on dopamine metabolism in mesolimbic and nigrostriatal pathways of male rat brain. Brain Res 218:189–206.

Aloisi AM, Della Seta D, Ceccarelli I, Farabollini F. 2001. Bisphenol-A differently affects estrogen receptors-alpha in estrous-cycling and lactating female rats. Neurosci Lett 310:49–52.

Arnett J. 1992. Reckless behavior in adolescence: a developmental perspective. Dev Rev 12:339–373.

Arnold AP, Gorski RA. 1984. Gonadal steroid induction of structural sex differences in the central nervous system. Annu Rev Neurosci 7:413–442.

Atanassova N, McKinnell C, Turner KJ, Walker M, Fisher JS, Morley M, et al. 2000. Comparative effects of neonatal exposure of male rats to potent and weak environmental estrogens on spermatogenesis at puberty and the relationship to adult testis size and fertility: evidence for stimulatory effects of low estrogen levels. Endocrinology 141:3898–3907.

Bardo MT, Donohew RL, Harrington NG. 1996. Psychobiology of novelty seeking and drug seeking behavior. Behav Brain Res 77:23–43.

Bardo MT, Neisewander JL, Pierce RC. 1988. Novelty-induced place preference behavior in rats: effect of opiate and dopaminergic drugs. Pharmacol Biochem Behav 32:683–689.

Beatty WW. 1979. Gonadal hormones and sex differences in non-reproductive behaviors in rodents: organizational and activational influences. Horm Behav 12:112–163.

Becker JB. 1999. Gender differences in dopaminergic function in striatum and nucleus accumbens. Pharmacol Biochem Behav 64:803–812.

Benus RF, Koolhas JM, Van Oortmerssen GA. 1987. Individual differences in behavioral reaction to a changing environment in mice and rats. Behaviour 100:105–122.

Bizot J, Le Bihan C, Puech AJ, Hamon M, Thiebot M. 1999. Serotonin and tolerance to delay of reward in rats. Psychopharmacology 146:400–412.

Bradshaw CM, Szabadi E. 1992. Choice between delayed reinforcers in a discrete-trials schedule: the effect of deprivation level. Q J Exp Psychol B 44:1–16.

Brotons JA, Olea-Serrano FF, Villalobos M, Pedraza V, Olea N. 1995. Xenoestrogens released from lacquer coatings in food cans. Environ Health Perspect 103:608–612.

Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. 1992. Evidence for the decreasing quality of semen during the past 50 years. Br Med J 305:609–612.

Christian M, Gillies G. 1999. Developing hypothalamic dopaminergic neurones as potential targets for environmental estrogens. J Endocrinol 160:R1–R6.

Cyr M, Calon F, Morissette M, Di Paolo T. 2002. Estrogenic modulation of brain activity: implications for schizophrenia and Parkinson's disease. J Psychiatry Neurosci 27:12–27.

Dessì-Fulgheri F, Porrini S, Farabollini F. 2002. Effects of perinatal exposures to bisphenol-A on play behavior of female and male juvenile rats. Environ Health Perspect 110(suppl 3):403–407.

Dluzen DE. 2000. Neuroprotective effects of estrogen upon the nigrostriatal dopaminergic system. J Neurocytol 29:387–399.

Euvrard C, Oberlander C, Boissier JR. 1980. Antidopaminergic effect of estrogens at the striatal level. J Pharmacol Exp Ther 214:179–185.

Evenden J. 1999. Varieties of impulsivity. Psychopharmacology 146:348–361.

Evenden JL, Ryan CN. 1996. The pharmacology of impulsive behaviour in rats: the effects of drugs on response choice with varying delays of reinforcement. Psychopharmacology 128:161–170.

———. 1999. The pharmacology of impulsive behaviour in rats VI: the effects of ethanol and selective serotonergic drugs on response choice with varying delays of reinforcement. Psychopharmacology 146:413–421.

Exner M, Clark D. 1993. Behaviour in the novel environment predicts responsiveness to amphetamine in the rat: a multivariate approach. Behav Pharmacol 4:47–56.

Farabollini F, Porrini S, Della Seta D, Bianchi F, Dessì-Fulgheri F. 2002. Effects of perinatal exposure to bisphenol A on sociosexual behavior of female and male rats. Environ Health Perspect 110(suppl 3):409–414.

Farabollini F, Porrini S, Dessì-Fulgheri F. 1999. Perinatal exposure to the estrogenic pollutant bisphenol A affects behavior in male and female rats. Pharmacol Biochem Behav 64:687–694.

Fink G, Sumner BE, Rosie R, Grace O, Quinn JP. 1996. Estrogen control of central neurotransmission: effect on mood, mental state, and memory. Cell Mol Neurobiol 16:325–344.

Fink G, Sumner B, Rosie R, Wilson H, McQueen J. 1999. Androgen actions on central serotonin neurotransmission: relevance for mood, mental state and memory. Behav Brain Res 105:53–68.

Fisher JS, Turner KJ, Brown D, Sharpe RM. 1999. Effect of neonatal exposure to estrogenic compounds on development of the excurrent ducts of the rat testis through puberty to adulthood. Environ Health Perspect 107:397–405.

Guillette LJJ, Pickford DB, Crain DA, Rooney AA, Percival HF. 1996. Reduction in penis size, and testosterone concentrations, in juvenile alligators living in a contaminated environment. Gen Comp Endocrinol 101:32–42.

Howdeshell KL, Hotchkiss AK, Thayer KA, Vandenbergh JG, Vom Saal FS. 1999. Exposure to bisphenol-A advances puberty. Nature 401:763–764.

Hruska RE, Pitman KT. 1982. Distribution and localization of estrogen-sensitive dopamine receptors in the rat brain. J Neurochem 39:1418–1423.

Hughes RN. 1968. Behavior of male and female rats with free choice of two environments differing in novelty. Anim Behav 16:92–96.

Hutchison JB. 1997. Gender-specific steroid metabolism in neural differentiation. Cell Mol Neurobiol 17:603–626.

Kabbaj M, Devine DP, Savage VR, Akil H. 2000. Neurobiological correlates of individual differences in novelty-seeking behavior in the rat: differential expression of stress-related molecules. J Neurosci 20:6983–6988.

Kaylor WM Jr, Song CH, Copeland SJ, Zuspan FP, Kim MH. 1984. The effect of estrogen on monoamine systems in the fetal rat brain. J Reprod Med 29:489–492.

Kelly PH, Seviour PW, Iversen S. 1975. Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res 94:507–522.

Kim HS, Han SY, Yoo SD, Lee BM, Park KL. 2001. Potential estrogenic effects of bisphenol-A estimated by *in vitro* and *in vivo* combination assays. J Toxicol Sci 26:111–118.

Lilienthal H, Weinand-Harer A, Winteroff H, Winneke G. 1997. Effects of maternal exposure to 3, 3´, 4, 4´-tetrachlorobiphenyl or propylthiouracil in rats trained to discriminate

apomorphine from saline. Toxicol Appl Pharmacol 146:162–169.

Linnoila M, Virkkunen M, Scheinin M, Nuutila A, Rimon R, Goodwin FK. 1983. Low cerebrospinal fluid 5-hydroxyindolacetic acid concentrations differentiates impulsive from non-impulsive violent behavior. Life Sci 33:2609–2614.

Logue AW. 1988. Research on self-control: an integrated framework. Behav Brain Sci 11:665–709.

Macrì S, Adriani W, Chiarotti F, Laviola G. 2002. Risk-taking during exploration of a plus-maze is greater in adolescent than in juvenile or adult mice. Anim Behav 64:541–546.

McClusky NJ. 1981. Sexual differentiation of the central nervous system. Science 211:1294–1303.

McEwen BS. 1992. Steroid hormones: effect on brain development and function. Horm Res 37:1–10.

Meaney MJ, Stewart J. 1981. A descriptive study of social development in the rat (*Rattus norvegicus*). Anim Behav 29:34–45.

Menniti FS, Baum MJ. 1981. Differential effects of estrogen and androgen on locomotor activity induced in castrated male rats by amphetamine, a novel environment, or apomorphine. Brain Res 216:89–107.

Misslin R, Herzog F, Koch B, Ropartz P. 1982. Effects of isolation, handling and novelty on the pituitary-adrenal response in the mouse. Psychoneuroendocrinology 7:217–221.

Misslin R, Ropartz P. 1981. Effects of metamphetamine on novelty-seeking behavior by mice. Psychopharmacology 75:39–43.

Olea N, Pulgar R, Perez P, Olea-Serrano F, Rivas A, Novillo-Fertrell A, et al. 1996. Estrogenicity of resin-based composites and sealants used in dentistry. Environ Health Perspect 104:298–305.

Osterlund MK, Halldin C, Hurd YL. 2000. Effects of chronic 17beta-estradiol treatment on the serotonin 5-HT (1A) receptor mRNA and binding levels in the rat brain. Synapse 35:39–44.

Osterlund MK, Hurd YL. 1998. Acute 17 beta-estradiol treatment down-regulates serotonin 5HT1A receptor mRNA expression in the limbic system of female rats. Brain Res Mol Brain Res 55:169–172.

Palanza P, Morley-Fletcher S, Laviola G. 2001. Novelty seeking in periadolescent mice: sex differences and influence of intrauterine position. Physiol Behav 72:255–262.

Panksepp J. 1981. The ontogeny of play in rats. Dev Psychobiol 14:327–332.

Peris J, Decambre CH, Coleman-Hardee ML, Simpkins JW. 1991. Estradiol enhances behavioral sensitization to cocaine and amphetamine-stimulated striatal [^{3}H]dopamine release. Brain Res 566:255–264.

Pierce RC, Crawford CA, Nonneman AJ, Mattingly BA, Bardo MT. 1990. Effect of forebrain dopamine depletion on novelty-induced place preference behavior in rat. Pharmacol Biochem Behav 36:321–352.

Rebec GV, Christiansen JRC, Guerra C, Bardo MT. 1997a. Regional and temporal differences in real-time dopamine efflux in the nucleus accumbens during free-choice novelty. Brain Res 776:61–67.

Rebec GV, Grabner CP, Johnson M, Pierce RC, Bardo MT. 1997b. Transient increases in cathecolaminergic activity in medial prefrontal cortex and nucleus accumbens shell during novelty. Neuroscience 76:707–714.

Renner MJ. 1990. Neglected aspects of exploratory and investigatory behavior. Psychobiology 18:16–22.

Richards JB, Mitchell SH, De Wit H, Seyden L. 1997. Determination of discount functions with an adjusting amount procedure in rats. J Exp Anal Behav 67:353–366.

Richards JB, Zhang L, Mitchell SH, De Wit H. 1999. Delay or probability discounting in a model of impulsive behavior: effect of alcohol. J Exp Anal Behav 71:121–143.

Robbins TW, Everitt BJ. 1996. Neurobehavioural mechanisms of reward and motivation. Curr Opin Neurobiol 6:228–236.

Rubin BS, Murray MK, Damassa DA, King JC, Soto AM. 2001. Perinatal exposure to low doses of bisphenol A affects body weight, patterns of estrous cyclicity, and plasma LH levels. Environ Health Perspect 109:675–680.

Rubinow DR, Schmidt PJ, Roca CA. 1998. Estrogen-serotonin interactions: implications for affective regulation. Biol Psychiatry 44:839–850.

Sagvolden T. 2000. Behavioral validation of the spontaneously hypertensive rat (SHR) as an animal model of attention-deficit/hyperactivity disorder (AD/HD). Neurosci Biobehav Rev 24:31–39.

Sagvolden T, Aase H, Zeiner P, Berger DF. 1998. Altered reinforcement mechanisms in attention-deficit hyperactivity disorder: hyperactivity may be required. Behav Brain Res 94:61–71.

Sagvolden T, Sergeant JA. 1998. Attention deficit/hyperactivity disorder: from brain dysfunctions to behaviour. Behav Brain Res 94:1–10.

Soubrié P. 1986. Reconciling the role of central serotonin neurones in human and animal behavior. Behav Brain Sci 9:319–364.

Spear LP, Brake SC. 1983. Periadolescence: age-dependent behavior and psychopharmacological responsivity in rats. Dev Psychobiol 16:83–109.

Staton DM, Solomon PR. 1984. Microinjections of D-amphetamine into the nucleus accumbens and caudate-putamen differentially affects stereotypy and locomotion in the rat. Physiol Psychol 12:159–162.

Stewart PW, Reihman J, Lonky E, Darvill T, Pagano J. 2001. The use of signal detection and operant DRL performance as measures of impulsivity/ response inhibition in children exposed to PCBs. Neurotoxicology 22:867–888.

Terranova ML, Laviola G, Alleva E. 1993. Ontogeny of amicable social behavior in the mouse: gender differences and ongoing isolation outcomes. Dev Psychobiol 26:467–481.

Terranova ML, Laviola G, De Acetis L, Alleva E. 1998. A description of the ontogeny of mouse agonistic behavior. J Comp Psychol 112:3–12.

Thiebot MH, Le Bihan C, Soubrié P, Simon P. 1985. Benzodiazepines reduce the tolerance to reward delay in rats. Psychopharmacology 86:147–152.

Tohei A, Suda S, Taya K, Hashimoto T, Kogo H. 2001. Bisphenol A inhibits testicular functions and increases luteinizing hormone secretion in adult male rats. Exp Biol Med 226:216–221.

Vom Saal FS, Cooke PS, Buchanan DL, Palanza P, Thayer KA, Nagel SC, et al. 1998. A physiologically-based approach to the study of bisphenol A and other estrogenic chemicals on the size of reproductive organs, daily sperm production, and behavior. Toxicol Ind Health 14:1–21.

Vom Saal FS, Nagel SC, Palanza P, Boechler M, Parmigiani P, Welshons WV. 1995. Estrogenic pesticides: binding relative to estradiol in MCF-7 cells and effects of exposure during fetal life on subsequent territorial behavior in male mice. Toxicol Lett 77:343–350.

Williams K, Fisher JS, Turner KJ, McKinnell C, Saunders PT, Sharpe RM. 2001a. Relationship between expression of sex steroid receptors and structure of the seminal vesicles after neonatal treatment of rats with potent or weak estrogens. Environ Health Perspect 109:1227–1235.

Williams K, McKinnell C, Saunders PT, Walker M, Fisher JS, Turner KJ, et al. 2001b. Neonatal exposure to potent and environmental oestrogens and abnormalities of the male reproductive system in the rat: evidence for importance of the androgen-oestrogen balance and assessment of the relevance to man. Hum Reprod Update 7:236–247.

Wise RA. 1996. Neurobiology of addiction. Curr Opin Neurobiol 6:243–251.

Wolff MS, Toniolo PG, Leel EW, Rivera M, Dubin N. 1993. Blood levels of organochlorine residues and risk of cancer. J Natl Cancer Inst 85:648–652.

Zimmermann A, Stauffacher M, Langhans W, Würbel H. 2001. Enrichment-dependent differences in novelty exploration in rats can be explained by habituation. Behav Brain Res 121:11–20.

Zuckerman M. 1994. Behavioral Expressions and Biosocial Bases of Sensation Seeking. Cambridge, UK:Cambridge University Press.