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To breed or not to breed? Empirical evaluation of drug effects in adolescent rats

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ABSTRACT

The recent upsurge of research on adolescent rats raises the issue of the extent to which different methods of rodent procurement might affect results. Here, we examined the effects of acute and repeated dosing with two antipsychotics, haloperidol and clozapine, and Δ^9-tetrahydrocannabinol [Experiments 1 and 2, respectively] in adolescent rats of both sexes that differed in shipping status (i.e., shipped from a commercial breeder at weaning or bred in-house). In each experiment, test drugs produced effects that were characteristic for their respective classes in previous studies with adult rodents. Both haloperidol and clozapine produced catalepsy and haloperidol decreased locomotion in shipped and bred rats of both sexes, with sensitization to haloperidol-induced catalepsy developing with repeated dosing. The most prominent between-status difference in this experiment was greater sensitivity of the shipped rats to haloperidol-induced changes in locomotor activity over a wider dose range, an effect that was especially evident in females. In Experiment 2, vehicle levels of motor activity were decreased in bred rats (which did not occur in Experiment 1), resulting in flattening of the Δ^9-tetrahydrocannabinol dose–effect curve for this measure in bred rats of each sex. Acutely, Δ^9-tetrahydrocannabinol produced antinociception, hypothermia and catalepsy in both groups of rats, with tolerance developing after repeated dosing. Status-related differences were sex-dependent. Whereas bred female rats were more sensitive to Δ^9-tetrahydrocannabinol's antinociceptive effects, shipped male rats were more sensitive to its antinociceptive effects as well as to its hypothermic and cataleptic effects. Together, the results of these descriptive experiments suggest that between-status differences tend to be quantitative rather than qualitative. Further, these results suggest that careful attention to issues related to rodent procurement during adolescence is warranted and may help to account for divergent findings in different labs.

Rodents that are used for developmental psychopharmacology research typically are obtained by one of the several ways: they are shipped from a commercial breeder, they are born to pregnant dams that have been shipped or they are bred in animal facilities located at the institution where they will be used. The decision of whether or not to breed rodents for research is not a trivial one, as maintaining a breeding colony may require considerable time, space and effort as well as dedicated personnel. On the other hand, the shipping process introduces issues that are not faced when using rodents that are bred in-house. For example, the dams and/or pups may experience stress due to the shipping process, particularly if shipping occurs during vulnerable periods (Ogawa et al., 2007). Even among developmental psychopharmacologists, shipping stress is not always controlled, as timed pregnant dams are frequently shipped rather than impregnated in institution's animal facility.

Another consequence of shipping is the potential loss of control over litter effects. Among scientists that examine development in rodent models, the term 'litter effect' refers to spontaneous or treatment-induced similarities among animals born in the same litter. The effect is a consequence of using multiparous species (e.g., rodents) as research subjects and has the potential to affect the outcome of a study if left uncontrolled (Haseman and Hogan, 1975; Zorrilla, 1997). Perhaps the most effective method of controlling this possible confound is to use litter as the unit of analysis; i.e., only a single animal from each litter is used in an experiment or the mean value of the dependent measure for the entire litter is counted as a single observation for the purposes of data analysis (Festing, 2006; Spear and File, 1996). In addition to being a threat to the internal and external validity of the experiment, use of more than a single observation from a litter results in a violation of independence of observations that serves as the foundation for

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Abbreviations: %MPE, percentage of maximal possible antinociceptive effect; PN, postnatal day; Δ^9-THC, Δ9-tetrahydrocannabinol.

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most statistical analysis techniques, including analysis of variance (Holson and Pearce, 1992).

In contrast with the concern about litter and shipping stress in developmental studies, research designs with adult rodents rarely consider these issues, albeit most studies allow a period of acclimation between arrival of shipped animals and their entry into a study. An informal survey of published psychopharmacology studies in major journals performed by the authors revealed that adult rats are almost always purchased and shipped from a commercial breeder. Indeed, most rodents used in a long-term study such as drug discrimination or one that employs operant responding were originally ordered at the same time from the same vendor. Although this approach probably results in animals that are from the same litter, the major point here is that verification of litter status under these conditions is impossible and the effects of this common practice are undetermined. Hence, issues that are of paramount importance to developmental psychopharmacologists involved in pre- and perinatal research are virtually ignored by psychopharmacologists that do not have a developmental concentration.

A recent trend in psychopharmacology is increased interest in possible differences in drug effects in adolescent versus adult rats. Adolescence in rodents is typically defined as the period of time [approximately postnatal day (PN) 28–42] during which immature rodents exhibit behavior patterns that are characteristic of the transition from juvenile to adult in mammalian species, including preference for novelty, increased risk taking, increased interaction with peers, and development of sexual maturity (Spear, 2000). Accompanying these outward behavioral changes are dramatic pruning and widespread re-organization of central neurons (Spear, 2000). Since many researchers in this area have previously concentrated solely on studies with adult animals, the degree of control exercised over litter effects and shipping stress differs considerably across laboratories. This study represents an examination of the empirical consequences of ignoring the impact of these factors and their interactions with drug actions in adolescent animals. To this end, we compared the pharmacological effects of the antipsychotics haloperidol and clozapine (Experiment 1) and Δ^9-tetrahydrocannabinol (Δ^9-THC) [Experiment 2] in adolescent rats that were bred and born in our animal facility to their effects in adolescent rats that were shipped shortly after weaning. The two experiments were designed and conducted independently; hence, procedural parameters were not identical across experiments. Selection of drugs was based upon funding considerations, as this investigation was initiated as a result of issues raised in research funded by two separate grants. Nevertheless, choice of drugs from at least two distinct classes provides greater opportunity for generalization of results. In addition, the effects of antipsychotics and cannabinoids have not been well characterized in adolescents, despite considerable therapeutic use of antipsychotics in human children and adolescents and abuse of marijuana by adolescents. Pharmacological assessment assays were chosen to correspond with those known to be sensitive to antipsychotics (e.g., motor behavior) and cannabinoids [e.g., battery of pharmacological tests used to characterize this class of compounds (Martin et al., 1991)].

1. Methods

1.1. Subjects

Two categories of adolescent rats were used for each experiment (i.e., antipsychotics and Δ^9-THC). The first group consisted of male and female Long–Evans rats that were ordered from a commercial breeder (Harlan, Dublin, VA) as juveniles aged PN 22–25. Upon arrival, these rats were housed in clear plastic cages in same-sex pairs. Rats were allowed at least 3 days to habituate to the vivarium environment before treatment as suggested by the results of several studies (Capdevila et al., 2007; Landi et al., 1982). During this time period, rats were left undisturbed in their home cages. In the first group (shipped rats), rats in the different dose groups for each drug were randomly chosen from the rats received in the shipment. In order to maintain similarity to acquisition conditions in most studies with adult rodents, no other controls (e.g., for litter, degree of shipping stress) were instituted. The second category of adolescent rats consisted of rats that were born in our animal room facility. In order to obtain rats for this group, adult female Long–Evans rats (Harlan, Dublin, VA) were impregnated by adult male Long–Evans rats (Harlan, Dublin, VA). After breeding, dams were individually housed in clear plastic cages with plenty of sawdust bedding available in each cage for nesting. The dams were left undisturbed except for providing food, water, and fresh bedding until they gave birth (PN0). Pups were sexed and culled to no more than 10 pups per litter. No other restraints were placed on size of litter. Pups that were not used in this study were used in other independent studies. They remained with their dams until weaning at PN21. On PN21, pups were separated from the dam and were pair-housed with a same-sex rat from another litter that had also been bred and born in our animal facility. In this second group (in-house rats), no more than one male and one female rat pup were chosen from each litter and were randomly assigned to one of the different dose groups for one of the test drugs. Data for males and females were never compared statistically; hence, choosing one pup of each sex did not violate the assumptions of ANOVA for independent samples. All rats in both categories (shipped vs. bred) were housed in a temperature-controlled (20–22 °C) environment with a 12-h light–dark cycle (lights on at 7 a.m.). In addition, all rats remained in their home cages when not being tested and had free access to food and water throughout the experiment. The studies reported in this manuscript were carried out in accordance with guidelines published in guide for the care and use of laboratory animals (National Research Council, 1996.) and were approved by our Institutional Animal Care and Use Committee.

1.2. Apparatus

Clear plastic rat cages (22.5 cm width × 44 cm length × 20 cm height) were housed in sound-attenuating cabinets and were used as locomotor chambers. Each cabinet contained up to 12 chambers, with a maximum of 2 per shelf. Chambers did not contain bedding and were wiped with alcohol solution between sessions. Sessions occurred in darkness (i.e., with the cabinet doors closed). A cage rack system with 4 × 8 equally spaced photocell beams on the *X*- and *Y*-axes (Lafayette Instrument, Lafayette, IN) was placed around each chamber (4.5 cm from bottom of cage) and locomotor activity was measured as total number of beam breaks for the entire session. The bar apparatus that was used to measure catalepsy consisted of a 280-mm bolt (10 mm diameter) that was attached to a frame by eyebolts at a height of 98 mm. Each bar apparatus was housed in its own box that was open in the front for experimenter access. Testing in the catalepsy assay was performed in ambient fluorescent lighting conditions in the lab. A tail flick analgesia meter (Columbus Instruments, Columbus, OH) and a Traceable7 digital thermometer (Control Company, Friendsville, TX) were used to measure antinociception and rectal temperature, respectively. The 8 V (6 amp) high intensity light of the tail flick apparatus was set at medium (intensity = 13 in range of 1–25) and the light was turned off after a maximum of 10 s, regardless of whether or not the rat moved its tail.

1.3. Experimental procedures

1.3.1. Experiment 1: antipsychotics

Male and female adolescent rats from both groups were randomly assigned to receive daily injections of saline, haloperidol (0.03, 0.1 or 0.3 mg/kg) or clozapine (3, 10 or 30 mg/kg) for 10 consecutive days, PN30–PN39, inclusive. On each day of testing, rats were transported to the laboratory in their home cages at least 30 min before drug administration. Subsequently, each rat was injected intraperitoneally with saline or with their assigned dose of haloperidol or clozapine. Rats were assessed in the bar catalepsy test three times (30, 45 and 60 min) after the initial injection. At each assessment time point, the front paws of the rat were placed on the bar apparatus for 5-min. The total amount of time (in s) that both of the rat's front paws remained in contact with the bar during the 5-min session was recorded. If both of the rat's paws dropped from the bar, they were re-positioned as before. The session timer was stopped during the brief time needed for re-positioning. If the rat voluntarily removed its paws from the bar 10 times during the session, the session was stopped and amount of time on bar was recorded as 0. Invariably, this situation occurred during the first minute of the session and was almost always associated with saline treatment. After the final 5-min bar test (i.e., 65 min after injection), rats were placed into the locomotor chamber for a 15-min session. Locomotor activity was measured as total number of beam breaks for the entire session. After the session, rats were returned to their home cages and transported back to the animal facility. This procedure was repeated daily for 10 days. In order to complete all testing during the short duration of adolescence in rats (approximately 2 weeks), habituation to the locomotor chambers prior to drug administration was not included in the study design. Timing and sequence of tests and injections and handling procedures for the adolescent rats from each group were identical during the 10-day assessment period.