Early sexual experience alters voluntary alcohol intake in adulthood

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HIGHLIGHTS

- Sexual experience during adolescence but not adulthood increases alcohol self-administration.
- Sexual experience increased anxiety- and depressive-like behaviors.
- Adolescent experiences have enduring effects on adult phenotype.

STEROID HORMONES SIGNALING BEFORE AND AFTER BIRTH SEXUALLY DIFFERENTIATES NEURONAL CIRCUITRY. ADDITIONALLY, STEROID HORMONES RELEASED DURING ADOLESCENCE CAN ALSO HAVE LONG LASTING EFFECTS ON ADULT BEHAVIOR AND NEURONAL CIRCUITRY. AS ADOLESCENCE IS A CRITICAL PERIOD FOR THE ORGANIZATION OF THE NERVOUS SYSTEM BY STEROID HORMONES IT MAY ALSO BE A SENSITIVE PERIOD FOR THE EFFECTS OF SOCIAL EXPERIENCE ON ADULT PHENOTYPE. OUR PREVIOUS STUDY INDICATED THAT EARLY ADOLESCENT SEXUAL ACTIVITY ALTERED MOOD AND PREFRONTAL CORtical MORPHOLOGY BUT TO A MUCH SMALLER EXTENT IF THE SEXUAL EXPERIENCE HAPPENED IN LATE ADOLESCENCE. IN HUMANS, BOTH SUBSTANCE ABUSE DISORDERS AND MOOD DISORDERS GREATLY INCREASE DURING ADOLESCENCE. AN ASSOCIATION AMONG BOTH AGE OF FIRST SEXUAL ACTIVITY AND AGE OF PUBERTY WITH BOTH MOOD AND SUBSTANCE DISORDERS HAS BEEN REPORTED WITH ALCOHOL BEING THE MOST COMMONLY ABUSED DRUG IN THIS POPULATION. THE GOAL OF THIS EXPERIMENT WAS TO DETERMINE WHETHER SEXUAL EXPERIENCE EARLY IN ADOLESCENT DEVELOPMENT WOULD HAVE ENDURING EFFECTS ON ADULT AFFECTIVE AND DRUG-SEEKING BEHAVIOR. COMPARED TO SEXUALLY INEXPERIENCED HAMSTERS AND THOSE THAT EXPERIENCED SEX FOR THE FIRST TIME IN ADULTHOOD, ANIMALS THAT MATED AT 40 DAYS OF AGE AND WERE TESTED EITHER 40 OR 80 DAYS LATER SIGNIFICANTLY INCREASED DEPRESSIVE-BUT NOT ANXIETY-LIKE BEHAVIORS AND INCREASED SELF-ADMINISTRATION OF SACCHARINE-SWEETENED ETHANOL. THE RESULTS OF THIS STUDY SUGGEST THAT AN ISOLATED, THOUGH HIGHLY RELEVANT, SOCIAL EXPERIENCE DURING ADOLESCENCE CAN SIGNIFICANTLY ALTER DEPRESSIVE-LIKE BEHAVIOR AND ALCOHOL SELF-ADMINISTRATION IN ADULTHOOD.

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Sexual development of the brain and behavior has long been conceptualized as a two-stage process wherein steroid hormone exposure during the prenatal and early postnatal period permanently organizes neural circuitry and establishes the manner in which these circuits will respond to steroid hormones later in life [24]. As originally conceived, this organizational-activational hypothesis has provided deep insights into explaining the role of hormones in sexually differentiated behavior; more recently, this hypothesis has been expanded to include persistent effects of steroid hormones outside of the early life critical periods [5,30,32]. As an example, exposure to androgenic steroids during the peripubertal period appears necessary for the full expression of male-typical sex behavior in adulthood [20,27–29]. Specifically, if male hamsters are deprived of androgens during puberty, male-typical levels of sexual or aggressive behavior in adulthood do not occur even when treated with testosterone [28]. Similarly, if male Siberian hamsters mate with a sexually receptive conspecific during adolescence, a manipulation that increases circulating testosterone, adult affective behavior, gene expression, and neuronal morphology is altered in adulthood [21]. Additionally, many of these effects were recapitulated by exogenous testosterone administration [21]. Adolescence is the developmental period that includes onset of puberty and subsequent sexual and neurological development [29]. Puberty begins in the brain with increased production and release of gonadotropin releasing hormone (GnRH) [29,31]. Importantly, during this period there is significant maturation of the nervous
system in general and the prefrontal cortex in particular [14,23]. For instance, there is a marked increase in the volume of gray matter, the numbers of synapses, and overall myelination in addition to greater pruning of prefrontal synapses [33,36,40]. Additionally, this period is marked by an increase in the dopaminergic input to the prefrontal cortex, as well as in dopamine receptor density and transporter activity [15,34]. Importantly, sex steroid hormones including testosterone can modulate many of these processes [18]. One potential side effect of this rapid neuronal development is that environmental stimuli, (including those that alter HPG axis physiology) during this period may have long lasting and far-reaching consequences on adult phenotype [1].

In humans, both substance abuse disorders and mood disorders greatly increase during adolescence [11]. An association among both age of first sexual activity and age of puberty with both mood and substance disorders has been reported [3,19,37]. Alcohol is the most frequently used and abused drug among adolescents, and it is the leading cause of mortality and morbidity in this age group; more than all other drugs collectively [22]. Our previous study indicated that adolescent sexual activity altered mood and prefrontal cortical morphology in adulthood apparently by increasing circulating testosterone (and potentially estrogens via aromatization) during this developmental epoch but to a much smaller extent if the sexual experience happened in late adolescence [21]. Therefore we hypothesized that early, but not later, adolescent sexual experience, which could potentially recapitulate the effects of early puberty, would increase voluntary alcohol intake and induce a depressive- and anxiety-like phenotype in adult Siberian hamsters.

1. Materials and methods

1.1. Animals

Siberian hamsters (Phodopus sungorus) used in this study were bred in our colony at The Ohio State University from a wild-bred stock originally obtained from Dr. K. Wynne-Edwards (Kingston, Ontario, Canada). Hamsters were housed in polystyrene cages (28 × 17 × 12 cm) with a nestlet and 1 cm of corn cob bedding. Hamsters were weaned at approximately 21 days in a long photoperiod (16:8 LD; with lights–off at 15:00 Eastern Standard Time [EST]) and housed within this room for the duration of the study. All hamsters had ad libitum access to food (Harlan Teklad Rodent Diet 8640, Indianapolis, IN, USA) and filtered tap water, except where experimental protocol dictated otherwise. Animal rooms were held at constant temperature and humidity (21 ± 2 °C and 50 ± 10%, respectively). All procedures were conducted in accordance with the US National Institute of Health’s Guide for the Care and Use of Laboratory Animals, The Ohio State University Institutional Animal Care and Use Committee, and the international ethical standards described previously by Portaluppi et al. [25].

At 40 or 80 days of age males were paired with an ovariec-tomized hormone-primed female (see below) or kept isolated (control condition). The females were removed after 6 h and mating behavior was recorded to ensure that copulation took place. Hamsters were provided with a sexual experience at 40 or 80 days of age, and then were tested 40 or 80 days later (40 × 40 vs. 40 × 80 vs. 80 × 40 vs. 80 × 80 groups). All behavioral testing was conducted between 15:00 and 18:00 h and hamsters were each given 30 min to habituate to the test room before initiation of testing. Tests were performed in the following order: (1) elevated-plus maze, (2) forced swim test, then (3) ethanol intake test (Figs. 1 and 2).

1.2. Sexual experience

The stimulus females were introduced to the males and allowed to copulate for a maximum of 6 h. Sexual behavior was induced between intact males and ovariec-tomized females. Female OVX hamsters were implanted with a 5 cm long estrogen capsule 2 weeks prior to the beginning of experiments. OVX females were brought into behavioral estrus by subcutaneous injections of progesterone 6 h prior to being sexually paired with a male. Bouts of mating behavior were conducted in a rectangular box measuring 50 × 75 × 50 cm (D × W × H), the front wall of which was transparent. Sexual contact was monitored and videotaped in the dark under red light, and tests were conducted between 15:00 and 21:00 h and recorded on-video tape to ensure that copulation had occurred.

Fig. 1. Adolescent sexual activity alters behavioral parameters (recapitulated). Effects of adolescent sexual activity on behavioral responses (mean ± SEM) in the behavioral measure. (A) Total number of arm entries in the testing arena. (B) Percentage of time spent in the open arm of the elevated plus maze. (C) Percentage of time spent floating in the forced swim test. Bars sharing the same letters are not statistically different from each other. Control: no sexual experience; 40 × 80: mated at 40 days tested 80 days later; 40 × 40: mated at 40 days and tested 40 days later; 80 × 40 mated at 80 days and tested 40 days later; 80 × 80 mated at 80 days and tested 80 days later.
2. Behavioral tests

2.1. Tests of anxiety-like responses

2.1.1. Elevated plus maze

At 80, 120, and 160 days of age Siberian hamsters were tested in the elevated plus maze. The EPM test consisted of two open and two closed 6 cm wide arms in a plus-sign configuration 1 m off the floor. The closed arms were enclosed by 15 cm tall black Plexiglas. All arms were covered with contact paper to prevent the hamsters from sliding off, and all surfaces were wiped with 70% alcohol between animals. Each hamster was released into one of the closed arms and allowed to move freely on the maze for a 5 min testing period that was videotaped from above the maze. Hamsters that fell off the maze into compartments below were placed back on the maze for the remainder of the testing period. An observer uninformed about experimental conditions scored the videotapes with The Observer software (Version 5, Noldus Software, Setauket, NY) for (a) percentage of entries scored into open arms (b) and total entries into all arms. Hamsters were considered to have entered an arm when all four paws crossed onto an arm of the maze.

2.2. Tests of depressive-like responses

2.2.1. Forced swim test

Hamsters were examined for cessation of attempting to escape water by placing them in 17 cm of room-temperature water (22 ± 1°C) in a cylindrical tank (24 cm diameter; 53 cm height) with opaque walls. Swimming behavior was videotaped for 7 min and scored by an uninformed observer with The Observer software (Noldus Corp., Leesburg, VA, USA) to determine (a) vigorous swimming (i.e., climbing or scratching directed at the wall of the tank and horizontal movement in the tank), and (b) non-vigorous swimming (i.e., minimal movement required to maintain head above the surface of the water).

2.3. Consummatory behavior

2.3.1. Alcohol consumption

Hamsters self-administered ethanol using a sucrose-substitution procedure that provides ad libitum access to a sipper tube containing 2 or 10% ethanol. Initially, two sipper tubes were introduced into the home cage, one tube containing ethanol solution in 2% sucrose in water and the other tube containing water. Daily measurements were made in the middle of the light period by reading fluid volume to the nearest 0.2 mL. The positions of the tubes was switched every 24 h to control for side preferences. Ethanol solutions were tested for 10 days: during the first five days, bottles were filled with 3% ethanol/2% sucrose, and during the next five days the bottles contained 10% ethanol/2% sucrose. Consumption of ethanol and water was measured daily at the same time. The tests were designed to avoid any possible “carry-over” effects from taste solutions on ethanol intake.

2.4. Statistical analyses

Data analyses were conducted using one-way analyses of variance (ANOVA) across groups. Alcohol intake was collapsed across the two testing periods. Data analysis was conducted with SPSS software, version 16.0 (SPSS, Chicago, IL). In all cases, mean differences were considered to be statistically significant when \( p < 0.05 \).

3. Results

Sexual experience during adolescence, but not in adulthood, induced a depressive-like phenotype in the Porsolt forced swim test. Specifically, there was an overall main effect of experimental group on number of floating bouts (\( p < 0.05 \)). That is, post hoc tests revealed that animals that experienced mating at 40 days of age floated significantly more often than all other groups. Similarly, there was an overall group effect on total time spent floating (\( p < 0.05 \)) that was again mediated by increased time in the two groups that mated at 40 days of age compared to all other groups (\( p < 0.05 \) in all cases).

In contrast, sexual experience, regardless of age increased the expression of anxiety-like behavior in the elevated plus maze. Specifically, there were overall group differences in time spent in the open arm (\( p < 0.05 \)) that was mediated by a reduction in open arm time in all sexually experienced groups (regardless of age) compared to sexually naive animals. Overall, total number of arm entries did not differ among groups (\( p > 0.05 \)).

Sexual experience during adolescence increased the amount of voluntary ethanol consumption. There was an overall main effect of group on ethanol intake at the lower concentration (3% EtOH; \( p < 0.05 \)) such that hamsters that experienced sex during early adolescence and were tested 80 days later consumed significantly more ethanol solution than did all groups (\( p < 0.05 \)). At the higher ethanol (10%) concentrations, there was also a significant group effect (\( p < 0.05 \)); intake was significantly elevated in the 40 × 80 group coupled with increased ethanol intake in the 40 × 40 and 80 × 40 groups (\( p < 0.05 \) in all cases) compared to sexually naive hamsters.
4. Discussion

Exposure to a highly salient social experience such as the opportunity to mate has significant and persistent effects on adult phenotype. Specifically, animals that mated at 40 days of age and were tested either 40 or 80 days later significantly increased depressive- but not anxiety-like behaviors and increased self-administration of saccharine-sweetened ethanol. These data are consistent with our previous report that early adolescent sexual experiences altered the morphology of prefrontal cortical neurons, increased proinflammatory cytokine gene expression, as well as induced depressive- and anxiety-like behavior in this species [21].

We chose to assess alcohol self-administration in this paradigm for several reasons. First, experimental and imaging studies have demonstrated that adolescence is a period of marked and dynamic changes in the morphology and physiology of the central nervous system [2,13,14]. Further, exposure to sex steroid hormones during this period appears to have long-term effects on adult phenotype [27,28]. Secondly, there is a wealth of data from both human and animal studies that adolescence is a period where the incidence of both substance abuse and psychiatric disorders greatly increases [2,9,10,34]. Additionally, the effects of adolescence appear to be magnified in individuals that undergo puberty earlier than their peers and begin engaging in sex at a younger age [16,38]. In this experiment, we sought to partially recapitulate the effects of early sexual experience and early puberty by exposing hamsters to a hormonally primed and thus sexually receptive female early during adolescence (40 days) or in early adulthood (80 days) and assessing their affective behavior and alcohol self-administration as adults.

Hamsters that mated at 40 days of age increased floating in the forced swim test compared to animals that mated at other ages (or were left isolated). Sexual experience, regardless of the age at which it occurred, significantly increased anxiety-like behaviors in the elevated plus maze (open arm entries) without altering total arm entries. In our previous study, we reported expression of proinflammatory cytokine IL-1β in the prefrontal cortex (PFC) of hamsters that mated at 40 days of age [26]. Proinflammatory cytokines rapidly and significantly induce depressive-like behaviors and may partially explain the depressive-like phenotype [26]. However, the source of cytokine production in the PFC remains unclear although there is mounting evidence for a role for immune system mediators in the actions of sex steroid hormones.

The large increase in incidence of substance use disorders at puberty and the emergence of sex differences at this time point strongly implicate sex steroid hormones in the development of addictive behaviors [2,4,8,9]. For instance, both estrogens and androgens can modulate the mesolimbic dopamine system and receptors for these steroid hormones are expressed by midbrain dopamine neurons and their afferents, a neurotransmitter system intimately involved in alcohol-related behaviors [6,7,12]. Further both androgens and estrogens increase alcohol self-administration in castrated male rodents and enhance mesolimbic dopamine release [6,35,39]. Presumably, enhanced androgen release during mating, when it coincided with the rapid neurodevelopment that occurs during adolescence produced persistent changes in reward processes such that hamsters self-administered increased quantities of ethanol and exhibited an increased depressive-like phenotype. Future studies should examine activity of the mesolimbic dopamine system following these manipulations and adolescent testosterone administration. Obviously alcohol self-administration is just one component of the development of alcohol addiction that is a complex process related to both positive and negative reinforcement and various neurochemical systems, but these data suggest a persistent phenotypic switch resulting from a single sexual experience [17].

Many psychosocial theories have postulated to explain the emergence of both affective and substance abuse disorders at puberty and the relationship among gender, age at puberty, and these outcomes [7,9]. The present data suggest that the social environment likely interacts with gonadal and neurodevelopment to influence mood and substance use outcomes. That is, early puberty may increase the probability that intercourse will occur at a younger age which may, in turn, influence gonadal steroid concentrations and potentially produce permanent changes to neurolucity that could influence the development of depression or substance use issues. These issues deserve further study. The results of this study suggest that an isolated, though highly relevant, social experience during adolescence can significantly alter depressivelike behavior and alcohol self-administration in adulthood.

References


