

1 Mutation in the vasopressin gene eliminates the sex difference in social reinforcement in  
2 adolescent rats.  
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30 Short Title: Vasopressin impacts sex differences in adolescent social reinforcement  
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30 Tables: 2 (1 in text, 1 supplemental)  
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59 27 **Abstract**  
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61 28 The neuropeptide, arginine vasopressin (AVP), is thought to contribute to sex differences in  
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63 29 normative and pathological social development by regulating social motivation. Recent studies  
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65 30 using Brattleboro rats that have a mutation in the *Avp* gene, however, have suggested that AVP  
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67 31 impacts adolescent social behaviors of males and females in a similar manner through actions  
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69 32 on behavioral state (i.e., arousal). In the present study, we made use of a recently developed  
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71 33 operant conditioning paradigm to test whether the chronic, lifelong AVP deficiency caused by  
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73 34 the Brattleboro mutation impacts the reinforcement value of social stimuli during adolescence.  
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75 35 Operant responding for access to a familiar conspecific was assessed in male and female  
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77 36 adolescent wild type (WT; normal AVP), heterozygous Brattleboro (HET), and homozygous  
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79 37 Brattleboro (HOM) rats. Following the social reinforcement test, rats were tested in the same  
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81 38 operant paradigm except that the social reinforcer was replaced with a light reinforcer to  
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83 39 determine whether effects of the Brattleboro mutation were specific to social stimuli or a general  
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85 40 characteristic of operant conditioning. WT males directed a greater proportion of their  
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87 41 responding toward the social and light stimuli than WT females; only males exhibited a  
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89 42 preference for these reinforcers over unreinforced ports. The sex difference in social  
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91 43 reinforcement was absent in HOM rats, whereas the sex difference in light reinforcement was  
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93 44 present in all genotypes. These data indicate that adolescent males are more sensitive to the  
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95 45 reinforcing properties of social and light stimuli, and that the sex difference in social, but not  
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97 46 light, reinforcement depends upon normal levels of AVP. These findings support the hypothesis  
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99 47 that AVP plays a critical role in sex differences in social development by acting on factors that  
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101 48 influence social motivation.  
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103 49  
104 50 **Keywords**  
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106 51 Brattleboro rat, vasopressin, sex differences, adolescence, social reinforcement, light  
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108 52 reinforcement  
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115 53 **1. Introduction**  
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117 54 Several neurodevelopmental disorders that impact social behavior exhibit striking sex  
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119 55 differences in incidence, severity, onset, and/or response to treatment. For example, autism  
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121 56 spectrum disorders are more prevalent in boys than girls (4.2 males: 1 female); and  
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123 57 schizophrenia, for which social withdrawal is a major symptom, is more prevalent (1.4 males: 1  
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125 58 female), develops earlier, and is more severe in males [1–3]. Sex differences in the  
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127 59 neurobiology of social development likely account for why one sex is more vulnerable, and the  
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129 60 other more resilient, to the social deficits that accompany neurodevelopmental disorders. Sex  
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131 61 differences in the brain and behavior arise from organizational and activational actions of  
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133 62 gonadal hormones, direct effects of genes on the sex chromosomes, and environmental factors  
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135 63 [reviewed in 4,5]. The downstream neurobiology on which these factors act to regulate sex  
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137 64 differences in social development, however, is not understood.  
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141 66 The neuropeptide, arginine vasopressin (AVP), has been implicated in several  
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143 67 neurodevelopmental disorders including autism spectrum disorders and schizophrenia [6–9].  
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145 68 AVP regulates several social and anti-social behaviors both in adulthood and during  
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147 69 development [reviewed in 10–13]. Notably, AVP's influence on social behaviors often differs  
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149 70 depending on sex. For example, AVP infusions into the anterior hypothalamus stimulate  
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151 71 aggression in male Syrian hamsters, but inhibit aggression in females [14–16]. During  
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153 72 development, ICV and septal infusions of a V1aR antagonist have opposite actions on the social  
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155 73 play of male and female juvenile rats [17,18], and septal infusions of AVP enhance social  
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157 74 recognition in female but not in male juvenile rats [19]. Hence, AVP is considered a prime  
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159 75 candidate substrate for regulating sex differences in social behavior and social development  
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161 76 [20].  
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171 78 The Brattleboro rat is a useful model for studying chronic, lifelong disruptions to AVP function.  
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173 79 Brattleboro rats have a single base-pair deletion in exon 2 of the *Avp* gene that disrupts the  
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175 production of AVP [21]. In this model, loss of AVP function at the level of the kidneys leads to  
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177 the development of diabetes insipidus [22]. Several social behaviors are also impacted,  
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179 presumably due to the loss of central AVP actions [23–25]. Few studies have tested both sexes  
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181 in the same experiment, but when comparing across studies adult male Brattleboro rats show  
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183 deficits in social discrimination, whereas adult female Brattleboro rats do not [26,27]. In juvenile  
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185 and adolescent rats, however, the Brattleboro mutation affects social interactions of males and  
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187 females in a similar manner – increases huddling and decreases social play and 50 kHz  
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189 ultrasonic vocalizations [28,29]. Hence, while acute intracranial pharmacological manipulations  
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191 of AVP are known to differentially impact male and female social behaviors, the data for chronic  
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193 AVP disruption are mixed.  
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198 91 AVP is thought to regulate social behavior through actions on social motivation, but few studies  
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200 directly test this conjecture. Complex behaviors can be influenced by many factors. Indeed,  
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202 adolescent Brattleboro rats do not exhibit deficits in a simple social approach test, but instead  
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204 exhibit a hypoaroused phenotype that is correlated with their decreased levels of social play  
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206 [29]. These findings raise the possibility that AVP influences social behavior through actions on  
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208 arousal. Recently, operant conditioning paradigms capable of testing social reinforcers have  
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210 been developed that allow for a direct assessment of social motivation and  
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212 reinforcement/reward value of social stimuli [30–33]. In the present experiment, we used one  
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214 such operant paradigm to test whether the Brattleboro mutation affects the social reinforcement  
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216 of adolescent male and female rats, and if so, whether it impacts the sexes in the same or  
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218 different manner. A previous study found that the Brattleboro mutation eliminated the sex  
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220 difference in a non-social learning task (extinction of a conditioned taste aversion) present in  
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222 adult Long Evans rats [34]. Hence, we also assessed operant responding to a light reinforcer,  
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227 104 which has been shown to function as a non-social reinforcer in operant paradigms [35–37], to  
228 105 assess whether effects seen in the present experiment are specific to social reinforcement or  
229 106 generalize to other types of operant conditioning.  
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235 108 **2. Materials and Methods**  
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237 109 *2.1. Subjects*  
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239 110 Experimental subjects were 14 wild type (WT; 6 female, 8 male) rats, 30 rats heterozygous for  
240 111 the Brattleboro mutation (HET; 14 female, 16 male), and 20 rats homozygous for the Brattleboro  
241 112 mutation (HOM; 10 female, 10 male) from our breeding colony, which was originally derived  
242 113 from HET rats obtained from the Rat Resource and Research Center (University of Missouri,  
243 114 Columbia, MO). All subjects were generated from HET male x HET female pairings in order to  
244 115 generate offspring of all three genotypes within the same litter. Experimental subjects were  
245 116 derived from 9 litters. All animals within each litter were used in experiments, except when a  
246 117 same-sex, same-genotype cagemate was not available at weaning for pair housing. This  
247 118 resulted in the following mean number of subjects for each sex/genotype per litter: 0.67 female  
248 119 WTs, 0.89 male WTs, 1.56 female HETs, 1.78 male HETs, 1.11 female-HOMs, and 1.11 male-  
249 120 HOMs. All rats were housed in plastic cages (44 cm X 22.5 cm X 20.5 cm) with wood shavings  
250 121 and maintained on a 12 h light/12 h dark cycle throughout the experiment. Food and water  
251 122 were available *ad libitum* and ambient temperature was maintained at 23°C. All experiments  
252 123 were in accordance with the *Guide for the Care and Use of Laboratory Animals* and were  
253 124 approved by the Animal Care and Use Committee at the University at Buffalo, State University  
254 125 of New York.  
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272 127 *2.2. Experimental Timeline*  
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274 128 Rats were genotyped on postnatal day (P)15 and subsequently weaned on P21 into same-sex,  
275 129 same-genotype pairs; day of birth = P0. Between P21-P23, rats were transferred from the  
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283 130 North Campus animal facility to the animal facility at the Clinical and Research Institute on  
284 131 Addictions (University at Buffalo, SUNY) for behavioral testing, at which point the time of lights  
285 132 off was shifted from 6PM to 7AM EST to facilitate testing during the dark phase. All rats were  
286 133 given at least 6 days to acclimate to the new building and altered light cycle. Rats were trained  
287 134 and tested on a social reinforcement task (training P28-P34; testing P35-P41) then a light  
288 135 reinforcement task (training P42-48; testing P49-P55; see methods below for details of the  
289 136 operant apparatus and procedure). Hence, training and testing occurred from early to late  
290 137 adolescence as defined by Vetter-O'Hagen and Spear [38]; early/mid adolescence = P28-42,  
291 138 late adolescence = P42-55. After testing was complete, rats were transferred back to the North  
292 139 Campus animal facility where they were individually housed and tested for 24-hour water intake  
293 140 measures between 10-13 weeks of age.

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307 142 *2.3. Genotyping Procedure*

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309 143 Rats were genotyped prior to weaning using the method developed by Paul et al. [28]. Between  
310 144 P13-P15, ear tissue was collected from rat pups using a sterile ear punch. Ear tissue was  
311 145 digested and DNA extracted using the REDExtract-N-Amp Tissue PCR Kit (SigmaAldrich). The  
312 146 DNA surrounding the single base pair Brattleboro deletion was amplified by PCR using the  
313 147 forward primer, GACGAGCTGGGCTGCTTC, and reverse primer,  
314 148 CCTCAGTCCCCACTTAGCC. The amplified DNA was then incubated at 37°C for 24 h with  
315 149 the restriction endonuclease, Bcg1 (New England BioLabs). Following incubation with the  
316 150 restriction endonuclease, samples were run on a 2% agarose gel using gel electrophoresis and  
317 151 genotype designations determined by assessing the number and weight of DNA bands: WT = a  
318 152 single 222 bp band; HOM = a single ~95 bp band; HET = two bands, one at 222 bp and one at  
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339 155 *2.4. Operant Testing Apparatus*  
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341 156 Social reinforcement was measured in a set of locally constructed operant chambers (Figure 1),  
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343 157 as previously published [31, for video, see supplemental material or <http://ratgenes.org/social->  
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345 158 reinforcement-monitor/]. The three-chamber apparatus was housed inside of a cooler (Model #  
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347 159 3000000187, Coleman, Wichita, KS), blocking external stimuli. The center of the test chamber  
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349 160 was made of a clear acrylic tube (diameter: 22.75 cm, height: 25.5 cm) and set on a grid floor  
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351 161 (1/8 inch stainless steel rods, 0.7 cm apart). Social stimulus chambers (diameter: 21.5 cm,  
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353 162 height: 16.5 cm) were located on the right and left sides of the test chamber. Lateral  
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355 163 observation ports made of 2-inch PVC pipe provided access between the test chamber and the  
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357 164 social stimulus chambers, allowing test and social stimulus rats to contact snouts and vibrissae,  
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359 165 as well as the passage of odor cues. The test chamber also had a center observation port  
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361 166 located on the far side of the chamber from the front face, capable of providing access to liquid  
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363 167 reinforcers; liquid reinforcers were not used in the present study, but the center port was  
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365 168 available as an unreinforced port. Left and right stimulus lights were located in the roof of the  
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367 169 test chamber above the social stimulus chambers. A ventilation fan was located on the back  
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369 170 wall of the enclosure. Air vents were placed in the bottom of the social stimulus chambers and  
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371 171 led to air holes in the bottom of the sound and light attenuating enclosures. A lid was placed on  
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373 172 the tops of the social stimulus chambers, ensuring that the ventilation fan would draw air  
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375 173 through the bottom of the social stimulus chambers into the observation port connected to the  
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377 174 central test chamber, thereby presenting the test rat with olfactory cues from stimuli placed into  
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379 175 the social stimulus chambers. Two obstruction bars (8-32 × 2.5 bolts placed 17.5 mm apart)  
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381 176 were placed in the PVC pipe between the test chamber and the social stimulus chambers to  
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383 177 prevent the test and social stimulus rats from escaping into the alternative chamber. Figure 1D  
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385 178 illustrates the sliding door that controls access of the test rat to the social stimulus chambers.  
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387 179 Infrared photo sensors bisected the observation port 17.5 mm from the test chamber wall and  
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389 180 detected snout pokes into the observation port. The swinging door was used to open or close  
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395 181 the passageway between the test and social stimulus chambers. The swinging door was  
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397 182 normally closed and was opened by operating a 24-volt rotating solenoid (Ledex H-1141-033,  
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399 183 Johnson Electric, Hong Kong). Operation of the solenoid opened the door and then held it open  
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401 184 against the stop, which allowed physical contact between the test and stimulus rats and the free  
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403 185 passage of odor cues. The photo detector circuit input and the solenoid output were connected  
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405 186 to a computer interface (Med Associates, St. Albans, VT) allowing the computer to control  
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407 187 access contingent upon snout poke responses.  
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411 189 *2.5. Operant Procedure*  
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413 190 A social stimulus (the cage-mate) was first placed into either the left or right stimulus chamber  
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415 191 (counterbalanced). Stimulus chambers were removable and could be placed on either side of  
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417 192 the apparatus. Separate chambers were designated for stimulus animals versus empty  
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419 193 chambers; stimulus animal chambers were never used as empty chambers and vice versa to  
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421 194 prevent accumulation of odors in the empty chambers. For each test rat, the same rat served  
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423 195 as the stimulus rat for all the test sessions. The test rat was placed into the center test  
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425 196 chamber, and the number of snout poke responses into the three observation ports during an  
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427 197 18-min test session was recorded. The opening of the sliding doors to all observation ports was  
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429 198 response-contingent according to a variable-interval (VI) 1 min schedule of reinforcement,  
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431 199 separate for each port. Each rat was given 1 week to train on this task then tested during the  
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433 200 second week. Training/testing sessions occurred three days per week (every other day) for a  
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435 201 total of six training/testing sessions. Data from the three test sessions for each rat were  
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437 202 combined and used for analysis. The order in which animals were tested and the side of  
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439 203 reinforcement were randomized. House light stimuli used in the light reinforcement task (see  
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441 204 below) were never turned on during the social reinforcement portion of the experiment.  
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451 206 Following social reinforcement training and testing, rats completed an additional two weeks of  
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453 207 training and testing using the same schedule except that the social stimulus was replaced with a  
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455 208 house light. The light reinforcement port and active house light were on the opposite side to the  
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457 209 prior social reinforcement port and social stimulus. The same VI 1 min schedule of  
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459 210 reinforcement was used. Once activated, the light remained on for 5 seconds. As with social  
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461 211 reinforcement, the last three test sessions were combined and used for analysis.  
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465 213 *2.6. Water Intake Measures*  
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467 214 We have previously validated our genotyping procedure using sequencing [28]. In the present  
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469 215 experiment, we further confirmed HOM genotype designations by assessing each animal's 24-h  
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471 216 water intake. Rats were individually housed and the weights of their water bottles were  
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473 217 recorded. The bottles were weighed again 24 and 48 hours later and the average 24-h  
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475 218 decrease in weight over this period was used as a measure of water intake, reported in milliliters  
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477 219 (1g water = 1ml water).  
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481 221 *2.7. Statistical Analyses*  
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483 222 Social and light reinforcement measures were calculated as the proportion of responses  
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485 223 directed at the reinforcer (reinforcer nose pokes/total nose pokes). Group differences in  
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487 224 reinforcement and total responses during the testing phase as well as during the first session of  
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489 225 the training phase were assessed using a 2 x 3 ANOVA, with Sex and Genotype as the  
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491 226 independent variables. Reinforcement across the 3 training sessions for each genotype was  
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493 227 analyzed using repeated-measures ANOVA, with Sex and Session as independent variables. If  
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495 228 main effects or interactions of the overall ANOVA were significant, *post hoc* tests were  
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497 229 conducted using Fisher's PLSD. Preference for and aversion to the social or light reinforcer  
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499 230 were determined by comparing the confidence intervals of reinforcement measures for each  
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501 231 experimental group with the proportion of responses expected by chance. Given that there  
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507 232 were 3 possible ports, a random distribution of port responses would result in 33% of responses  
508 233 directed toward each port, i.e. a proportion of 0.33 for each port. Hence, if the lower confidence  
509 234 interval for the reinforced port was greater than 0.33, a preference was assumed and if the  
510 235 higher confidence interval for the reinforced port was less than 0.33 than an aversion was  
511 236 assumed. Outliers were identified using the Box and Whiskers plot on SPSS, which defines  
512 237 outliers as measures greater than 1.5 times the interquartile range and were removed prior to  
513 238 conducting the overall ANOVA or assessing preference; see Supplemental Table for number of  
514 239 outliers within each group for each measure. Final sample sizes are indicated within the bars of  
515 240 each figure or in the figure legend. All analyses were conducted using SPSS v23.0 (IBM).  
516 241 Significance was assumed when  $P < 0.05$ .  
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518 242  
519 243 **3. Results**  
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521 244 *3.1. Lifelong AVP-deficiency eliminates the sex difference in social reinforcement*  
522  
523 245 During the testing phase, there was a significant main effect of Sex on social reinforcement  
524 246 ( $P < 0.05$ , ANOVA). This was due to WT males directing a higher proportion of responses  
525 247 toward the social reinforcer than WT females (Fig. 2A;  $P < 0.05$ , Fisher's PLSD). This sex  
526 248 difference, however, was not significant in HET rats ( $P = 0.19$ , Fisher's PLSD) and was absent in  
527 249 HOM rats ( $P = 0.90$ , Fisher's PLSD). WT, HET, and HOM males as well as HET and HOM  
528 250 females responded greater than chance for the social reinforcer indicating a preference for the  
529 251 social port over the unreinforced ports, a preference not present in WT females (Table 1). While  
530 252 the WT females did not prefer the social port, they also did not find it aversive (Table 1). Total  
531 253 responding during the social reinforcement test was not impacted by the sex or genotype of the  
532 254 animals (Fig. 2B;  $P > 0.21$ , main effect of Sex, main effect of Genotype, and the interaction  
533 255 between Sex and Genotype, ANOVA).  
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563 257 Analysis of the training phase revealed that the sex difference in social reinforcement was not  
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565 258 present upon first exposure to the operant procedure and there were no differences among the  
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567 259 genotypes (Fig. 3A;  $P>0.39$ , main effect of Sex, main effect of Genotype, and the interaction  
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569 260 between Sex and Genotype on session 1). However, a sex difference in social reinforcement  
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571 261 emerged in WT and HET animals over subsequent sessions (Fig. 3B,C;  $P<0.05$ , main effect of  
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573 262 Sex in WT animals, interaction between Sex and Session in HET animals, repeated-measures  
574  
575 263 ANOVA). In HET animals, the sex difference was significant on sessions 2 and 3 ( $P<0.05$ ,  
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577 264 Fisher's PLSD), whereas in WT animals the sex difference was significant on session 3 only  
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579 265 ( $P<0.05$ , Fisher's PLSD). As seen during the testing phase, there was no evidence of a sex  
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581 266 difference during the training phase in the HOM rats (Fig. 3D;  $P>0.31$ , main effect of Sex, main  
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583 267 effect of Session, and the interaction between Sex and Session, repeated measures ANOVA).  
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587 269 *3.2. Males, but not females, exhibit a preference for light reinforcement*  
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589 270 During the testing phase of light reinforcement, there was also a main effect of Sex, with males  
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591 271 directing a higher proportion of responses to the light reinforcer than females (Fig. 4A;  $P<0.05$ ,  
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593 272 ANOVA). In contrast to social reinforcement, however, this sex difference was present in all  
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595 273 genotypes ( $P<0.05$ , WT, HET, and HOM males versus WT, HET, and HOM females,  
596  
597 274 respectively, Fisher's PLSD). Furthermore, males, but not females, of all genotypes exhibited a  
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599 275 preference for the light port over the unreinforced ports (Table 1). Total responding during the  
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601 276 light reinforcement test was also impacted by sex, but in this measure females exhibited greater  
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603 277 responding than males (Fig. 4B;  $P<0.05$ , main effect of Sex, ANOVA). Although the mean total  
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605 278 responses of females was higher than that of males for each genotype, these differences fell  
606  
607 279 short of significance in the post hoc tests ( $P=0.11$ ,  $0.08$ , and  $0.10$  for WT, HET, and HOM  
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609 280 comparisons, respectively, Fisher's PLSD).  
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620 282 In contrast to that seen on the first day of social reinforcement training, there were group  
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622 283 differences on the first day of light reinforcement training. There was a significant interaction  
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624 284 between Sex and Genotype on the first session of the training phase (Fig. 5A;  $P<0.05$ , ANOVA).  
625  
626 285 This was due to male-specific differences across genotypes: lower light responding in HOM  
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628 286 males compared to HET and WT males ( $P<0.05$  for both comparisons, Fisher's PLSD).  
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630 287 Although there was a trend toward Sex differences in WTs (males > females) and HOMs  
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632 288 (females > males) on the first day of training, these comparisons did not reach significance  
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634 289 ( $P=0.09$  for WT males vs. WT females;  $P=0.07$  for HOM males vs. HOM females; Fisher's  
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636 290 PLSD). Note that the trend toward a sex difference on Session 1 for HOM rats reached  
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638 291 significance in the repeated-measures analysis due to the removal of 1 male that was an outlier  
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640 292 on session 3 (Fig. 5D;  $P<0.05$ , Fisher's PLSD). Analysis across the three training sessions  
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642 293 indicated that light responding for all groups was at their highest level on the first session (Figs.  
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644 294 5B-D). WT male and female rats maintained the same level of responding throughout the  
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646 295 training phase ( $P>0.54$ , main effect of Session and interaction between Sex and Session,  
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648 296 repeated-measures ANOVA). For HET and HOM rats, there was a female-specific decline in  
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650 297 light responding on session 2 that was maintained on session 3 ( $P<0.05$  for HET and HOM  
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652 298 comparisons of session 1 vs. session 2 and 3, Fisher's PLSD). The sex difference in light  
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654 299 responding seen in the testing phase (males > females) tended to be present in WT rats  
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656 300 throughout the training phase ( $P=0.055$ , main effect of Sex, Repeated-measures ANOVA), but  
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658 301 this was only significant on session 3 ( $P<0.05$ , Fisher's PLSD). For HET rats, this sex  
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660 302 difference emerged on session 2 ( $P<0.05$ , Fisher's PLSD), but was not significant on session 3  
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662 303 ( $P=0.16$ , Fisher's PLSD). In HOM rats, females initially exhibited higher light responding on  
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664 304 session 1 ( $P<0.05$ , Fisher's PLSD), but this sex difference was absent on sessions 2 and 3  
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666 305 ( $P>0.25$ , session 1 vs. session 2 and 3, Fisher's PLSD).  
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675 307 *3.3. Confirmation of HOM genotype designation by drinking phenotype*  
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677 308 As expected, all rats designated as HOM by our genotyping procedure drank markedly more  
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679 309 water than WT and HET rats (Fig. 6), confirming HOM genotype assignments.  
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683 311 **4. Discussion**  
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685 312 The present findings demonstrate that chronic disruption of AVP disrupts sex differences in  
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687 313 adolescent social behavior, specifically the sex difference in adolescent social reinforcement.  
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689 314 Male adolescent WT rats directed a greater proportion of responses toward the social stimulus  
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691 315 than female adolescent WT rats. Indeed, WT males showed a significant preference for the  
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693 316 social stimulus over the unreinforced ports, whereas WT females did not. This sex difference  
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695 317 depended on a functional *Avp* gene: it was inconsistent in HET rats (i.e., present at the end of  
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697 318 training, but not during testing) and was completely absent in HOM rats. HET rats exhibit a  
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699 319 partial reduction in AVP mRNA expression and pituitary protein content [39]. Hence, a partial  
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701 320 reduction in AVP appears sufficient to diminish the sex difference in adolescent social  
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703 321 reinforcement. These findings support the hypothesis that AVP plays an important role in sex  
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705 322 differences in adolescent social development.  
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709 324 To our knowledge, the present study is the first to demonstrate increased operant responding  
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711 325 for social stimuli in male compared to female adolescent rats. Adolescent male rats often (but  
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713 326 not always) exhibit higher levels of social interactions, particularly social play behavior, than  
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715 327 their female counterparts [40–42][but see 43,44]. The sex difference in social reinforcement of  
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717 328 WT rats seen in the present study adds to the small but growing literature indicating that the  
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719 329 greater levels of social interactions in adolescent male rats is due, in part, to a greater sensitivity  
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721 330 of males to the reinforcing/rewarding properties of social stimuli compared to females.  
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723 331 Adolescent males exhibit a greater conditioned place preference (CPP) than females to a  
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725 332 compartment previously paired with social interactions [45]. The sex difference in CPP to social  
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731 333 interaction depends on housing conditions, being present in single-housed, but not pair-housed  
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733 334 rats [45–47]. In contrast, we detected the sex difference in social reinforcement preference  
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735 335 even though rats were pair-housed throughout the experiment. This suggests that the greater  
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737 336 sensitivity of males to social reinforcement/reward is present in group-housed rats and that  
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739 337 operant paradigms may be more sensitive in detecting such preferences than CPP.  
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741 338  
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743 339 Although often assumed, few studies directly test whether AVP influences social behavior by  
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745 340 regulating social motivation or reinforcement/reward value of social stimuli. Increased social  
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747 341 motivation is typically inferred by shortened latencies to approach another individual or  
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749 342 increased time spent in social contact [48]. The operant conditioning paradigm used in the  
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751 343 present experiment allowed us to more directly assess the role of AVP in social reinforcement.  
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753 344 The present findings support the hypothesis that AVP influences social behavior by modulating  
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755 345 the reinforcement/reward value of social stimuli.  
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757 346  
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759 347 We have previously found that male and female adolescent Brattleboro rats exhibit 1) an  
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761 348 atypical social behavior profile characterized by decreased active social behaviors (e.g., social  
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763 349 play, 50 kHz ultrasonic vocalizations) and increased passive social behaviors (e.g., huddling)  
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765 350 and 2) a hypoaroused phenotype characterized by decreased locomotor activity in an open field  
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767 351 and decreased marble burying [28,29]. Notably, decreased behavioral arousal is correlated with  
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769 352 decreased social play suggesting that the more passive social behavior phenotype of  
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771 353 Brattleboro rats is due, in part, to AVP's actions on arousal [29]. Perhaps AVP's actions on  
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773 354 arousal impact males and females to influence active versus passive social behaviors similarly  
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775 355 in both sexes, whereas AVP's actions on social reinforcement differentially impact the sexes to  
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777 356 modulate sex differences in social behaviors. AVP also regulates other factors that likely impact  
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779 357 complex behaviors – circadian timing and social recognition [19,49–51]. Hence, it is becoming  
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787 358 clear that AVP acts through multiple mechanisms to influence social behavior (e.g., social  
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789 359 motivation, behavioral state, behavioral timing, and social memory).  
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793 361 The results of the social reinforcement test are unlikely to be the result of sex-specific effects of  
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795 362 the Brattleboro mutation on learning. This possibility is raised by studies demonstrating 1) sex  
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797 363 differences in the performance on several learning and memory paradigms [52,53] and 2) AVP  
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799 364 influences on both social and non-social learning tasks, including operant conditioning [54,55].  
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801 365 Similar to the present findings, a previous study found that the Brattleboro mutation eliminates  
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803 366 the sex difference in extinction of a conditioned taste aversion to a sucrose-lithium chloride  
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805 367 pairing [34]. Nevertheless, in the present study rats learned the social reinforcement task within  
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807 368 the first session, at which point their social responding did not differ between groups. Instead,  
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809 369 the sex difference emerged in WT and HET animals as the social responding of females  
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811 370 declined in later training sessions. These data suggest that the loss of the sex difference in the  
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813 371 social reinforcement in HOM rats seen in the present study is not due to effects of the  
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815 372 Brattleboro mutation on learning.  
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819 374 Loss of the sex difference in operant responding of Brattleboro rats was specific to the social  
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821 375 stimulus, with males of all genotypes exhibiting a greater proportion of responses for the light  
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823 376 stimulus than their female counterparts during the testing phase. This too argues against a  
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825 377 general effect of the Brattleboro mutation on operant responding in the current behavioral  
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827 378 paradigm. Caution is warranted here, however, because all rats had undergone social  
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829 379 reinforcement testing before being subjected to light reinforcement. Hence, it is possible that  
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831 380 the different results seen for social and light reinforcers in HOM rats are due to interactions  
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833 381 between AVP-deficiency and the order of testing, with animals more adapted to the apparatus  
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835 382 and testing procedures during light reinforcement. In addition, the differing ages or pubertal  
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837 383 stages of animals during social versus light reinforcement could have also influence the results.  
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843 384 Adolescence is a time of remarkable neural, behavioral, and reproductive development, with  
844 385 marked changes in social behavior and cognitive performance in both humans and rodents [56].  
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846 386 Pubertal factors, which differ markedly between early/mid-adolescence (when social  
847 387 reinforcement was tested) to late adolescence (when light reinforcement was tested) [38], could  
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849 388 impact learning and memory although this topic is understudied [57]. The most compelling data  
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851 389 indicate a role for pubertal status and pubertal hormones in PFC-dependent cognitive flexibility  
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853 390 [58,59]. Given that the number of AVP cells and fibers increases during adolescence [60,61],  
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855 391 one would predict that AVP-deficiency would have a greater effect in late as opposed to early  
856  
857 392 adolescence, which is opposite to that seen in the present study. Nevertheless, future studies  
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859 393 are needed to determine whether AVP's role in reinforcement conditioning changes across  
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861 394 adolescence or is impacted by testing experience. Assessment of the training phase for light  
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863 395 reinforcement was also less clear. As seen with the social reinforcement task, rats learned the  
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865 396 light reinforcement task within the first session. Unlike in the social reinforcement task,  
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867 397 however, sex differences in light reinforcement were significant (in HOM rats) or approached  
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869 398 significance (in WT rats) on this session, raising the possibility of learning differences in the light  
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871 399 reinforcement task among groups. Here too, the order of testing could have impacted the data  
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873 400 as animals were extinguishing the social reinforcement task while acquiring the light  
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875 401 reinforcement task.  
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879 403 HOM and WT rats differ in their performance on a visual attention test – lateralized reaction time  
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881 404 task to a house light stimulus [62]. Notably, genotype differences are dependent on the duration  
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883 405 of the house light, with WT rats outperforming HOM rats at short durations (~0.2s) and HOM rats  
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885 406 outperforming WT rats at longer durations (~2s). The authors attributed these findings to  
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887 407 genotype differences in attention rather than light perception because varying the brightness of  
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889 408 the house light had no effect on performance in either genotype. Importantly, WT and HOM rats  
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891 409 performed similarly when the house light was presented for 4s, which is similar to the duration of  
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899 410 the light stimulus in the present study (5s). Hence, it is unlikely that genotype differences in the  
900 411 perception of the house light influenced the light reinforcement in the present study.  
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902 412 Furthermore, genotype differences in light perception would not account for the presence or  
903 413 absence of sex differences within a given genotype.  
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909 415 Activity can also impact performance on operant responding [reviewed in 52], and previous  
910 416 studies have demonstrated sex differences in locomotor activity (females > males) as well as  
911 417 decreased locomotor activity in HOM Brattleboro rats [29,63–66]. However, in contrast to the  
912 418 effect on social reinforcement, the Brattleboro mutation impacts locomotor activity of adolescent  
913 419 males and females in a similar manner [29]. Furthermore, in the present study there were no  
914 420 sex or genotype differences in the total number of responses during the social reinforcement  
915 421 task and data were analyzed as proportion of responses directed toward the social stimulus,  
916 422 thereby controlling for any potential differences in activity. Hence, the present findings are more  
917 423 consistent with differences in goal-directed responses than in overall activity.  
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922 425 Sex differences in brain and behavior arise from organizational actions of prenatal, early  
923 426 postnatal, and pubertal gonadal hormones; activational actions of gonadal hormones; direct  
924 427 effects of genes on the sex chromosomes; and environmental factors [reviewed in 4,5]. The sex  
925 428 difference in adolescent social reinforcement is likely the result of several of these factors acting  
926 429 on AVP circuits, the primary candidate being the sexually dimorphic AVP pathway that  
927 430 originates from cells in the bed nucleus of the stria terminalis (BNST) and medial amygdala  
928 431 (MeA). Males of most species that have been assessed have greater numbers of AVP cells and  
929 432 more dense projections than females [reviewed in 67,68] due to interactions between  
930 433 organizational hormone actions, activational hormone actions, and direct effects of genes on the  
931 434 sex chromosomes [69–73]. Given that the BNST and MeA of rodents receive extensive  
932 435 chemosensory input, sex differences in this pathway could contribute to sex differences in social  
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955 436 behavior through differential modulation of chemosensory input [74]. Nevertheless, selective  
956 437 ablation of AVP cells in the BNST decreased same-sex social investigation by males, without  
957 438 altering their detection of social odors or habituation and discrimination of non-social odors [75].  
958  
959 439 These findings suggest that BNST AVP can act through non-chemosensory mechanisms to  
960 440 regulate sex differences in social behavior. The BNST/MeA pathway is thought to link brain  
961 441 areas that regulate social behavior (the Social Behavior Network) with the mesocorticolimbic  
962 442 dopamine system [12,76–78]. In juveniles/early adolescents, AVP manipulations in the septum,  
963 443 a projection area of BNST/MeA cells, modulate local dopamine release [79] and social play  
964 444 [17,18] in sex-specific ways. Furthermore, septal injections of the dopamine agonist,  
965 445 apomorphine, counteract the depressive effects of a vasopressin receptor 1a antagonist on play  
966 446 behavior [79]. These data suggest that vasopressin interacts with dopamine in the lateral  
967 447 septum to regulate sex differences in reward-associated social behaviors of juveniles/early  
968 448 adolescents. Future studies using social reinforcement paradigms are needed to determine  
969 449 whether this AVP/dopamine mechanism in the septum (or elsewhere) regulates sex differences  
970 450 adolescent social behaviors through sex-specific modulation of social motivation.  
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## 451 452 **5. Conclusions**

453 The present findings demonstrate that AVP plays a critical role in sex differences in adolescent  
454 social reinforcement. This adds to the growing list of social behaviors that are influenced by  
455 AVP during adolescence (e.g., social play, social recognition, 50 kHz USVs, huddling)  
456 [17,19,28,80]. Furthermore, it supports the hypothesis that AVP influences adolescent social  
457 behaviors, in part, by modulating the reinforcement/reward value of social stimuli. AVP has also  
458 been implicated in several neurodevelopmental disorders that impact social behavior [8,9,81–  
459 87]. Hence, understanding the neurobiology through which AVP influences social development  
460 is critical for our understanding of normative and pathological development, both of which  
461 exhibit striking sex differences in humans [1–3,56,88,89].

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1027 470 in the collection, analysis and interpretation of data; in the writing of the report; and in the  
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1029 471 decision to submit the article for publication.  
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1033 473 **Declarations of Interest**  
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1035 474 None  
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1039 476 **References**  
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1403 747 **Figure Legends**  
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1405 748 Figure 1. Pictures and illustration of the operant testing apparatus from the (A,B) side and (C)  
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1407 749 top view. (D) Observation port door that opens to the social stimulus or empty chamber.  
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1411 751 Figure 2. The Brattleboro mutation eliminates the sex difference in social reinforcement. (A)  
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1413 752 Mean ( $\pm$ s.e.) proportion of responses directed toward the social reinforcement port in wild type  
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1415 753 rats (WT), heterozygous Brattleboro rats (HET), and homozygous Brattleboro rats (HOM). (B)  
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1417 754 Mean ( $\pm$ s.e.) total responses directed toward the social reinforcement and unreinforced ports.  
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1419 755 \*Indicates significant sex difference within genotype (P<0.05, Fisher's PLSD).  
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1421 756  
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1423 757 Figure 3. Sex differences emerge in WT and HET rats, but not HOM rats, during the latter  
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1425 758 stages of the social reinforcement training phase. Mean ( $\pm$ s.e.) proportion of responses  
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1427 759 directed toward the social reinforcement port in WT, HET, and HOM rats on the (A) first training  
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1429 760 session and (B-D) across all training sessions. Sample sizes for panels B-D: WT females = 6,  
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1431 761 WT males = 8, HET females = 10, HET males = 16, HOM females = 9, HOM males = 10.  
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1433 762 \*Indicates significant sex difference within genotype (P<0.05, Fisher's PLSD). Abbreviations  
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1435 763 defined in Figure 1.  
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1439 765 Figure 4. Males show a greater preference than females for the light reinforcer, irrespective of  
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1441 766 genotype. (A) Mean ( $\pm$ s.e.) proportion of responses directed toward the light reinforcement port.  
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1443 767 (B) Mean ( $\pm$ s.e.) total responses directed toward the light reinforcement and unreinforced ports.  
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1445 768 \*Indicates significant sex difference within genotype (P<0.05, Fisher's PLSD). Inset of panel B  
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1447 769 illustrates the main effect of Sex on total number of responses (P<0.05, ANOVA). Abbreviations  
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1449 770 defined in Figure 1.  
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1459 772 Figure 5. Acquisition data for light reinforcement training. Mean ( $\pm$ s.e.) proportion of responses  
1460 773 directed toward the light reinforcement port in WT, HET, and HOM rats on the (A) first training  
1461 774 session and (B-D) across all training sessions. Sample sizes for panels B-D: WT females = 6,  
1462 775 WT males = 8, HET females = 13, HET males = 16, HOM females = 10, HOM males = 9.  
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1466 776 \*Indicates significant sex difference within genotype (P<0.05, Fisher's PLSD). #Indicates  
1467 777 significant difference between HOM male rats and males of other genotypes (P<0.05, Fisher's  
1468 PLSD). Abbreviations defined in Figure 1.  
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1471 778  
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1475 780 Figure 6. Boxplot of mean daily water intake of rats genotyped as WT, HET, and HOM rats (see  
1476 781 methods for genotyping details). The lower and upper ends of the boxes represent the first and  
1477 782 third quartile range, respectively. Lines within the boxes represent the median. Whiskers  
1478 783 represent the lowest and highest values within the group, excepting outliers. The single outlier  
1479 784 is indicated by a shaded circle. Abbreviations defined in Figure 1.  
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 1515 787 Table 1. Lower and upper confidence intervals for proportion responding to social and light  
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 1517 788 reinforcers  
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| Genotype | Sex     | Social Responding |          | Light Responding |          |
|----------|---------|-------------------|----------|------------------|----------|
|          |         | Lower CI          | Upper CI | Lower CI         | Upper CI |
| WT       | Females | 0.28              | 0.45     | 0.27             | 0.47     |
|          | Males   | 0.40*             | 0.61     | 0.42*            | 0.62     |
| HET      | Females | 0.35*             | 0.44     | 0.31             | 0.49     |
|          | Males   | 0.38*             | 0.53     | 0.45*            | 0.56     |
| HOM      | Females | 0.34*             | 0.57     | 0.32             | 0.41     |
|          | Males   | 0.38*             | 0.51     | 0.41*            | 0.54     |

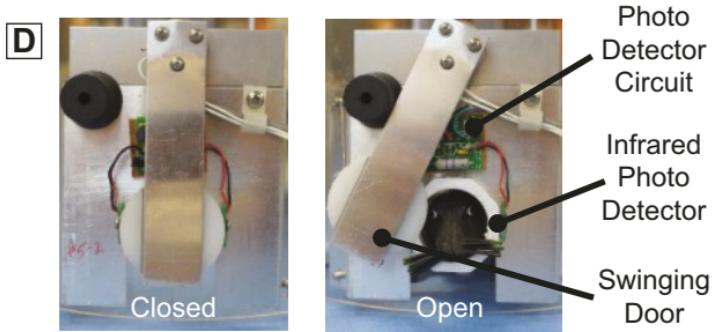
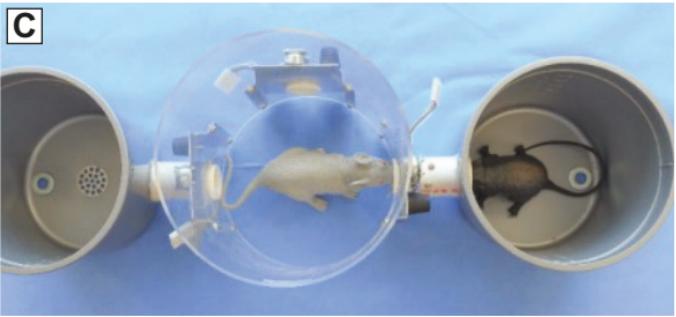
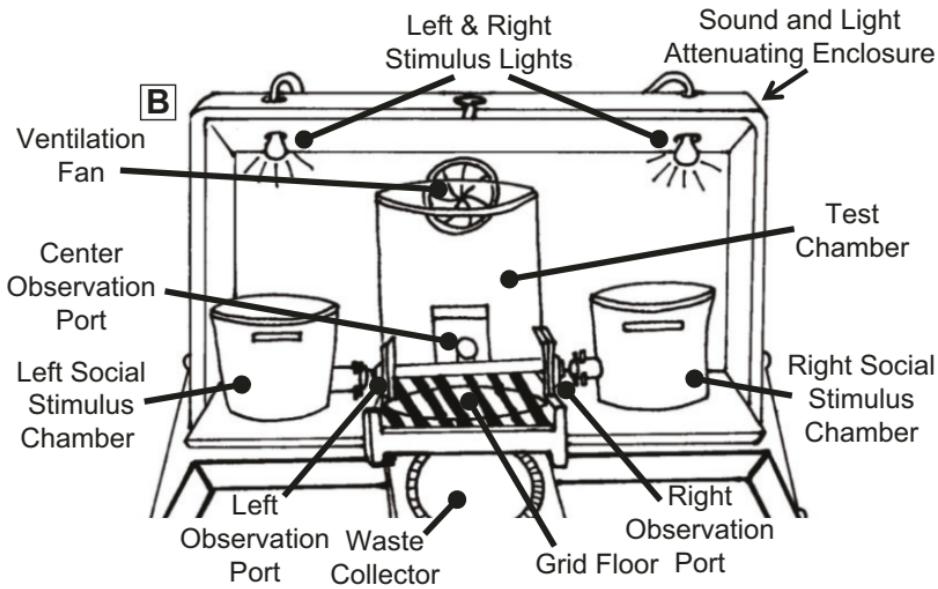
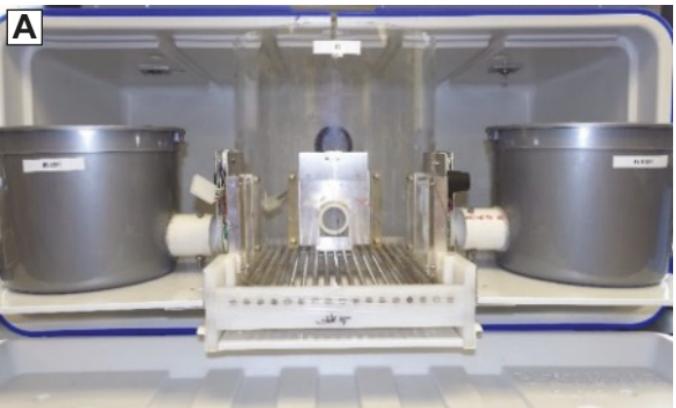
1519 789 \*Indicates significant preference for social or light reinforcer.  
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 1533 Supplemental Table. Outliers removed in each analysis.  
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| Genotype | Sex     | Social Reinforcement |                       |               | Total Responses | Light Reinforcement |                       |               | Total Responses |  |  |
|----------|---------|----------------------|-----------------------|---------------|-----------------|---------------------|-----------------------|---------------|-----------------|--|--|
|          |         | Social Responses     |                       |               |                 | Light Responses     |                       |               |                 |  |  |
|          |         | Training Session 1   | All Training Sessions | Testing Phase |                 | Training Session 1  | All Training Sessions | Testing Phase |                 |  |  |
| WT       | Females | 0                    | 0                     | 0             | 0               | 0                   | 0                     | 0             | 0               |  |  |
|          | Males   | 0                    | 0                     | 0             | 1               | 0                   | 1                     | 0             | 0               |  |  |
| HET      | Females | 0                    | 4                     | 0             | 1               | 1                   | 1                     | 0             | 1               |  |  |
|          | Males   | 0                    | 0                     | 0             | 1               | 0                   | 0                     | 0             | 0               |  |  |
| HOM      | Females | 0                    | 1                     | 0             | 1               | 0                   | 0                     | 1             | 1               |  |  |
|          | Males   | 0                    | 0                     | 0             | 0               | 1                   | 2                     | 0             | 0               |  |  |

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# Figure 1



# Figure 2

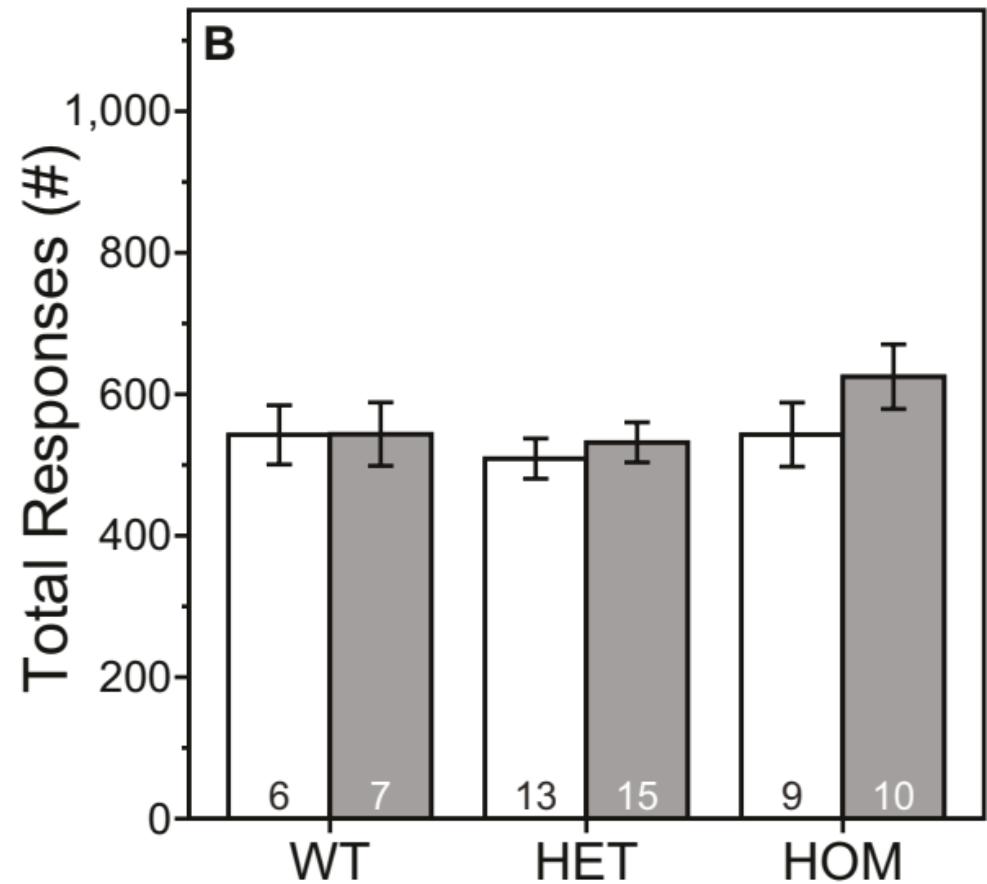
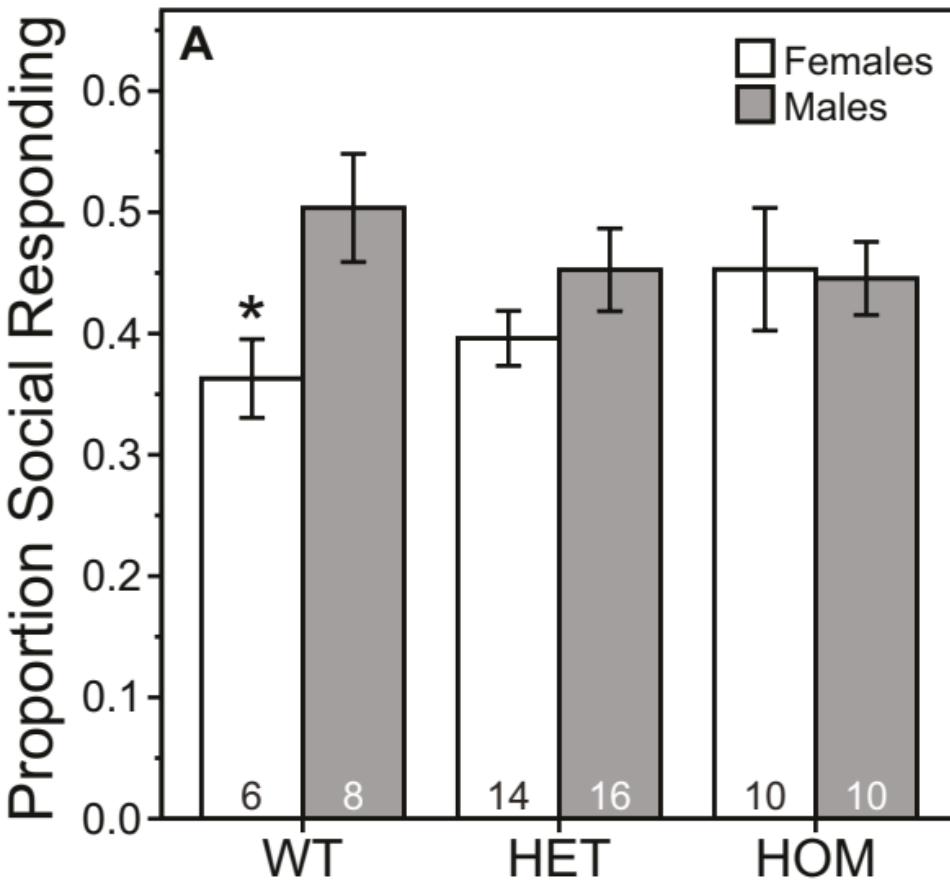
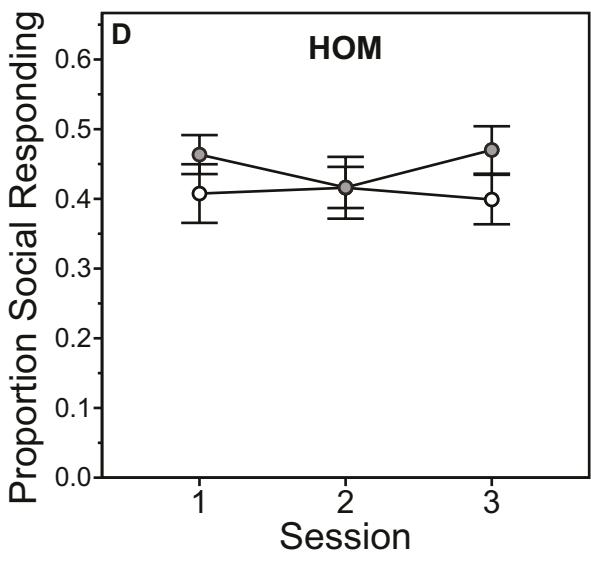
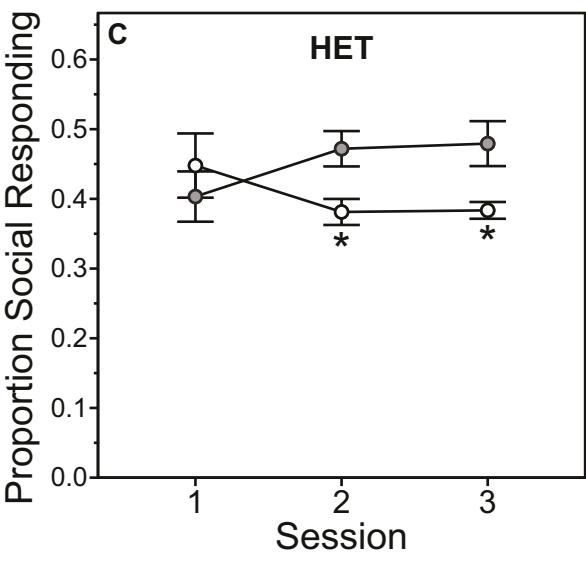
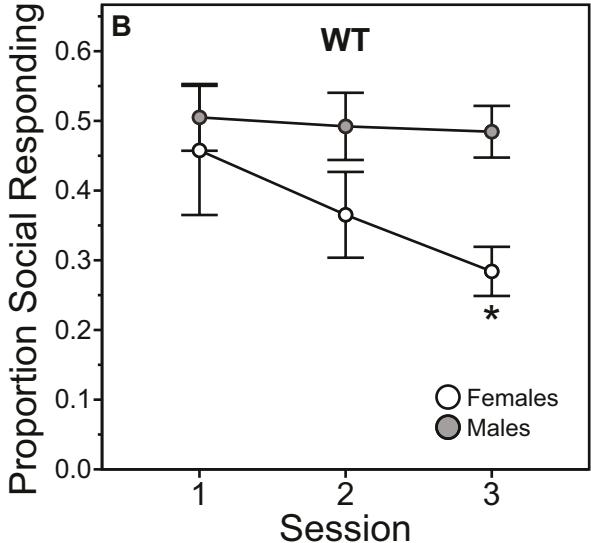
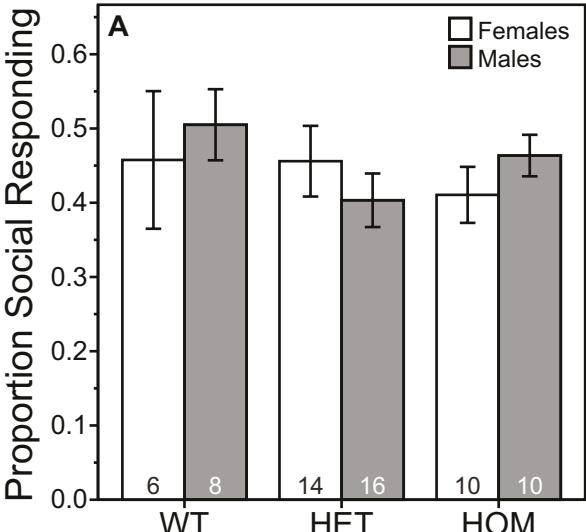


Figure 3



# Figure 4

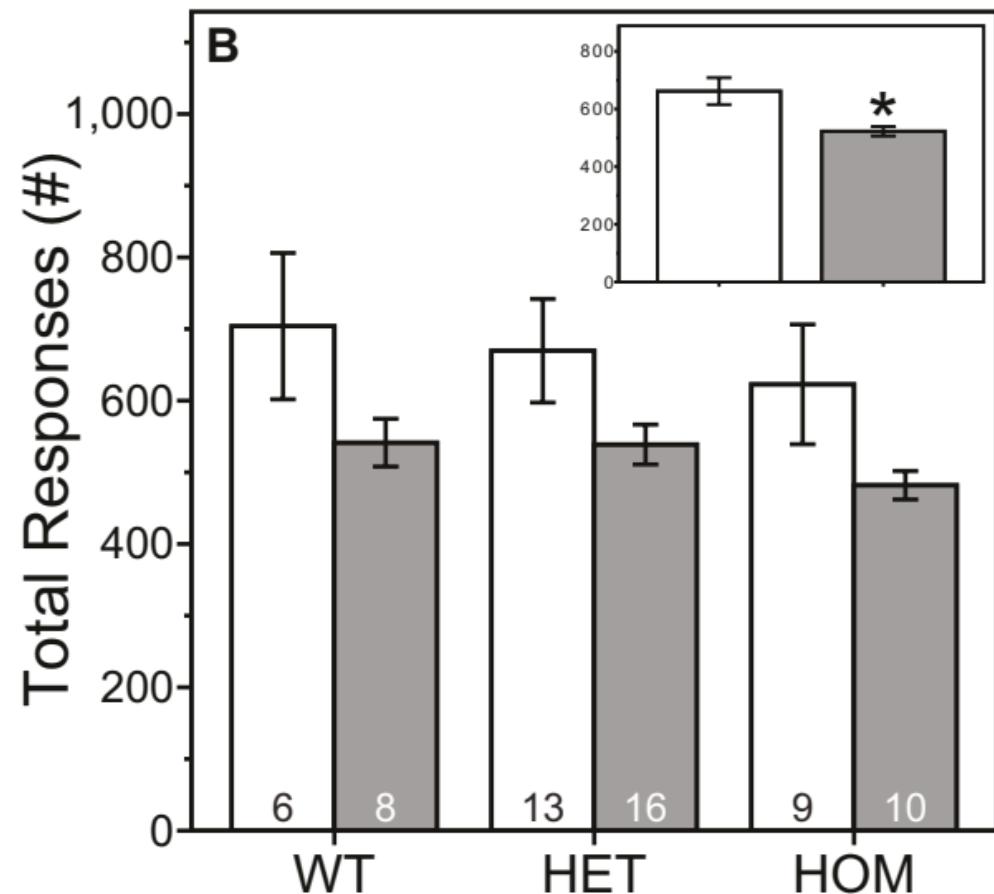
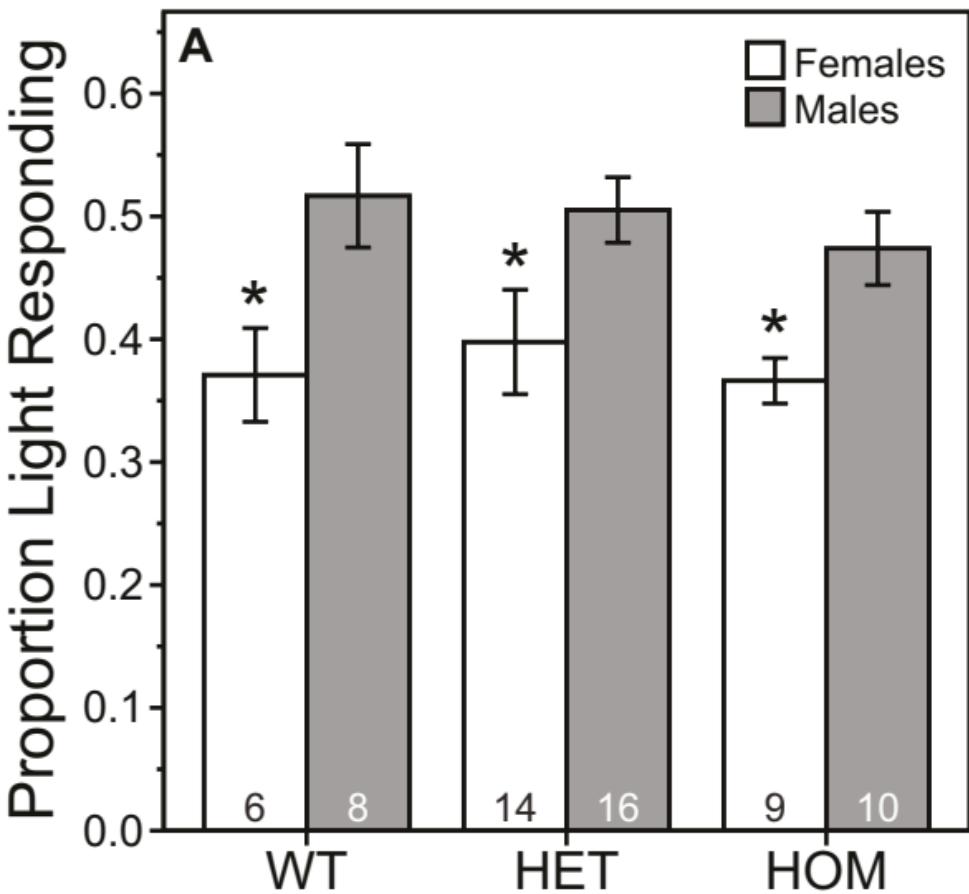
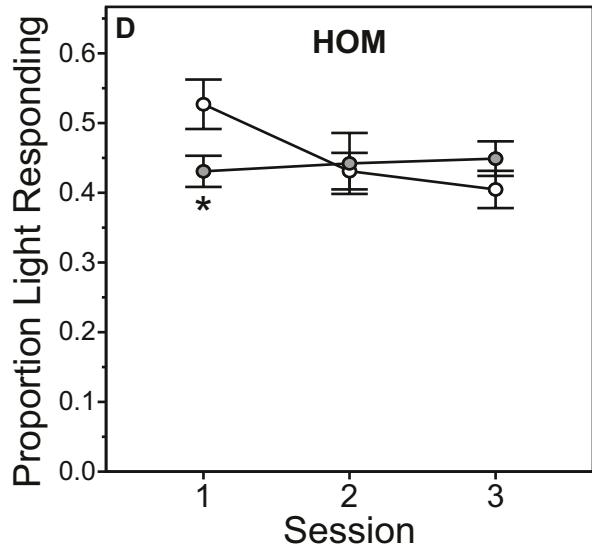
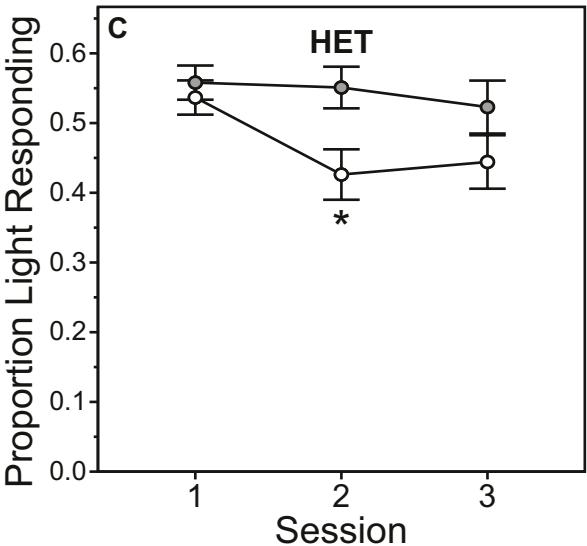
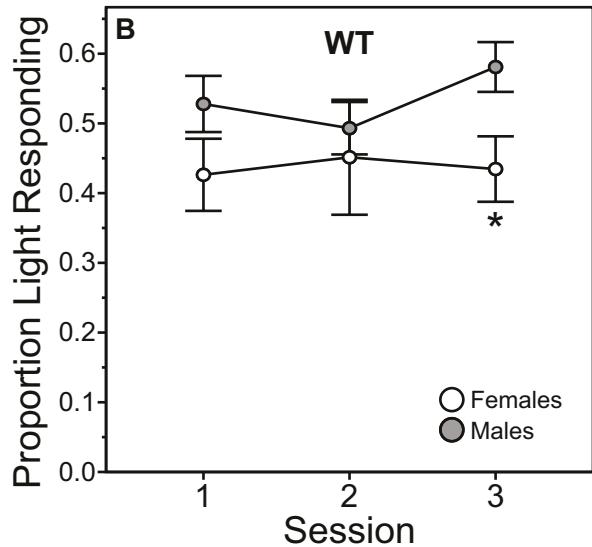
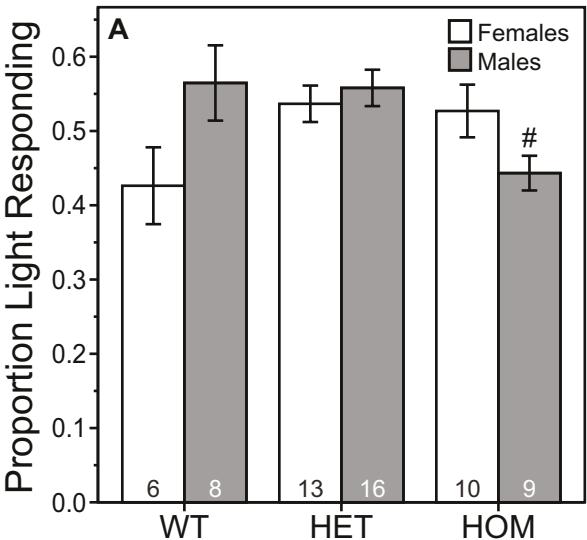


Figure 5



# Figure 6

