Measuring Virgin Female Aggression in the Female Intruder Test (FIT): Effects of Oxytocin, Estrous Cycle, and Anxiety

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Abstract

The costs of violence and aggression in our society have stimulated the scientific search for the predictors and causes of aggression. The majority of studies have focused on males, which are considered to be more aggressive than females in most species. However, rates of offensive behavior in girls and young women are considerable and are currently rising in Western society. The extrapolation of scientific results from males to young, non-maternal females is *a priori* limited, based on the profound sex differences in brain areas and functioning of neurotransmitters involved in aggression. Therefore, we established a paradigm to assess aggressive behavior in young virgin female rats, i.e. the female intruder test (FIT). We found that approximately 40% of un-manipulated adult (10–11 weeks old) female Wistar rats attack an intruder female during the FIT, independent of their estrous phase or that of their intruder. In addition, adolescent (7–8 weeks old) female rats selected for high anxiety behavior (HABs) displayed significantly more aggression than non-selected (NAB) or low-anxiety (LAB) rats. Intracerebroventricular infusion of oxytocin (OXT, 0.1 µg/5 µl) inhibited aggressive behavior in adult NAB and LAB, but not HAB females. Adolescent NAB rats that had been aggressive towards their intruder showed increased pERK immunoreactivity (IR) in the hypothalamic attack area and reduced pERK-IR in OXT neurons in the paraventricular hypothalamic nucleus compared to non-aggressive NAB rats. Taken together, aggressive behavior in young virgin female rats is partly dependent on trait anxiety, and appears to be under considerable OXT control.

Introduction

The prevalence, negative impact and costs of violence and aggression in our society have stimulated the scientific search for the predictors and causes of aggression, as well as possible treatments to reduce harmful violence. It has long been believed that aggression is mainly a problem among (especially young) males [1–3]. However, rates of violence and conduct disorder diagnoses in adolescent girls (aged 12–18 years) and young women (aged 18–24 years) are considerable [4,5] and have risen in Western society in recent years [6,7]. In addition to the damage done to the victims, the negative consequences of aggression extend to the perpetrators (both male and female): the display of increased aggressive behavior in childhood and adolescence is associated with substance abuse, lower socio-economic status, various social problems, and impaired physical health in adulthood [8–11].

These findings signal the need to increase the understanding of the neurocircuitry underlying aggression in both sexes, for example by making use of animal models of aggressive behavior. Although such translational studies have made great progress in the understanding of inter-male aggression in recent years, experiments focusing on females are lagging behind [12–14]. This

may be a problem, as results obtained in males are not necessarily representative for females. For example, the main hormones and neurotransmitters involved in inter-male aggression, namely testosterone, serotonin (5-HT), and vasopressin (AVP) [13,15–18], have profound sex-specific effects on social behaviors [19–23], including aggressive behavior [24–26]. In addition, many brain areas that are implicated in both inter-male and inter-female aggression, including the lateral septum (LS), the bed nucleus of the stria terminalis (BST), the anterior and ventromedial hypothalamus (AHA, VMH), the amygdala and the paraventricular hypothalamic nucleus (PVN) [15,27–31] are sexually dimorphic [32–33] and may control aggressive behavior in a sex-dependent manner. Due to the anticipated low levels of aggression in females from gregarious species, the (relatively rare) studies of female aggression have focused on lactating female rodents that show maternal defense [34–38], or on females from highly territorial rodent species such as Syrian hamsters (*Mesocricetus auratus*) and California mice (*Peromyscus californicus*) [39–41]. Although these studies have provided considerable knowledge on the neurobiology of aggression in lactating or territorial females, the extrapolation to non-maternal females of gregarious species is *a priori* limited: maternal aggression is under strong influence of parturition-associated neuroendocrine adaptations [42] and the

neurobiological control of offensive behavior in highly territorial species may differ from that of gregarious, group-living species such as rats and humans [43].

The main aim of the present study was, therefore, to establish and validate a suitable paradigm to measure aggression in adolescent and young adult (7–11 weeks old) virgin female rats, i.e. the female intruder test (FIT). We focused on this age group to match the age at which aggression peaks in girls [44] and at which effective treatment would be most beneficial. We anticipated that only a minority of the female residents would attack an intruder [45], putatively influenced by the estrous phase of residents and/or intruders as well as the level of innate anxiety of the residents. More precisely, we hypothesized that receptive (pro-estrous/estrous) residents would be less aggressive than non-receptive (met-estrous/di-estrous) residents [46] (but see [47]). Furthermore, we hypothesized that female Wistar rats selectively bred for high (HAB) or low (LAB) anxiety-related behavior (as measured in the elevated plus-maze (EPM) and other anxiety tests [48]) would display higher levels of aggression compared to non-selected (NAB) rats, as has been shown in male HAB and LAB rats [49].

In a follow-up experiment, we assessed the sensitivity of the FIT to pharmacological manipulation. Thus, we explored the behavioral effects of intracerebroventricular (ICV) infusion of oxytocin (OXT), a nonapeptide that has been shown to facilitate many social behaviors, both in rodents (social memory [50], social preference [51], pair bonding [52], maternal care and maternal aggression [42,53]) and in humans (face recognition [54], trust [55], empathy [56] and social reinforcement-induced learning [57]). OXT has been found to reduce aggression in female Syrian hamsters [58] and in male wild type Groningen rats selected for high aggression [59]. In addition, intranasal application of OXT reduced aggressive behavior in adult women with high levels of anxiety, but was ineffective in women with normal levels of anxiety [60].

Finally, we aimed to compare regional activation patterns in adolescent female NAB rats that had behaved either aggressively or non-aggressively toward an intruder in the FIT. Inspired by previous studies in male and female California mice [39,61,62], we analyzed the level of phosphorylated extracellular signal-regulated kinases (pERK) immunoreactivity (IR) in various brain areas known to be involved in aggressive behavior.

Methods

Ethics Statement

Experiments were approved by the Committee on Animal Health and Care of the Government of the Oberpfalz and are in accordance with the Guide for the Care and Use of Laboratory Animals produced by the National Institute of Health.

Animals

Experiments were carried out in female Wistar rats, which were bred in the animal facilities of the University of Regensburg, Germany and were either non-selected (NAB), or selectively bred for high (HAB) or low (LAB) anxiety-related behavior [49]. Intruder females were NAB Wistar rats obtained from Charles Rivers Laboratories (Sulzfeld, Germany). All rats were kept under controlled laboratory conditions (12:12 h light/dark cycle; lights off at noon, 21±1°C, 60±5% humidity, standard rat nutrition (RM/H, Ssniff Spezialdiäten GmbH, Soest, Germany) and water ad libitum) and housed in groups of 4–6 in standard rat cages (55×35×20 cm) with sawdust bedding until the start of the experimental procedures.

Male rats were not used in this set of experiments, but data from adult (16–22 week old) male Wistar rats (n = 108) generated in previous experiments were used here for illustrative purposes (see [49] and [63] for details on housing conditions).

Overview of Experiments (Fig. 1)

In exp. 1, general aggressive behavior and the link between aggression and the estrous cycle were assessed. Young adult NAB females (n = 69) underwent their first FIT at 10–11 weeks of age. The behavior of the females was compared with the behavior in the RI-test of 108 unmanipulated, naïve adult male NAB rats (a subset of the 176 males described in [63] for which attack frequencies were recorded). Vaginal smears were taken from both residents and intruders within one hour after completion of the FIT. This resulted in four experimental groups: receptive residents with receptive intruders (n = 15), receptive residents with non-receptive intruders (n = 20), non-receptive residents with receptive intruders (n = 11) and non-receptive residents with non-receptive intruders (n = 23).

In exp. 2, the link between aggressive behavior and anxiety was assessed in adolescent (7–8 weeks of age) female NAB (n = 23), HAB (n = 12) and LAB (n = 12) rats. The rats were tested for anxiety behavior on the EPM and, two days later, for aggressive behavior in the FIT.

In exp. 3, adult (10–11 weeks old) female NAB (n = 20), HAB (n = 20) and LAB (n = 20) females were tested in the FIT ("FIT 1"), placed back into their original group cages within 1 hour after FIT 1, and were left alone until surgery. Five days after FIT 1, the females underwent stereotaxic surgery for implantation of the ICV guide cannula and were allowed to recover in single observation cages for two nights. Females were divided into two treatment groups (VEH and OXT) per rat line based on their behavior in FIT 1, to ensure comparable baseline attack frequencies, attack latencies and percentage of aggressive behavior. Twenty min prior to FIT 2, the rats received an ICV infusion with either vehicle (VEH) or synthetic OXT. Group sizes were n = 10 per treatment and rat line.

In exp. 4, immunoreactivity of pERK alone and of pERK in OXT neurons was assessed in non-aggressive (n = 7) and aggressive (n = 7) adolescent female NAB rats – a subset of the rats tested in exp. 2. Females were selected for IHC analysis as follows: non-aggressive females had not attacked their intruder and had displayed aggressive behavior for less than 10% of the time in the FIT; aggressive females had attacked their intruder and had displayed aggressive behavior for more than 20% of the time. Both
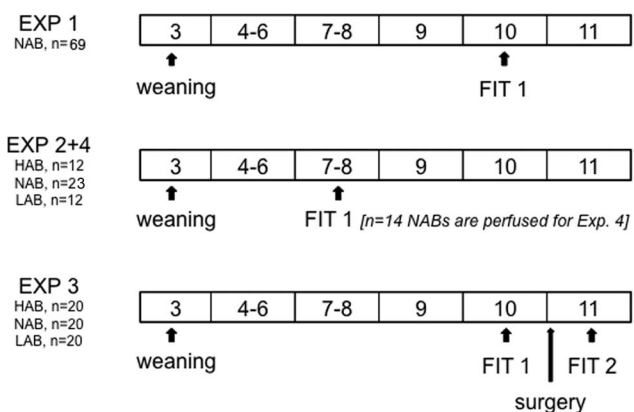

Figure 1. Timeline of experiments.
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groups contained approximately equal amounts of receptive/non-receptive residents and intruders.

Female Intruder Test (FIT)

The Female Intruder Test was adapted from the resident-intruder test (RI-test) in males as performed in our laboratory (see [63] for a detailed description). Similar to RI-tests, the FITs took place in the early dark phase (13.00–16.00 h) under dim red light conditions. Whereas adult male residents are typically housed with a female cage mate in an experimental observation cage (40×24×35 cm, Plexiglas walls) for ten days prior to the RI-test, in the adapted paradigm the experimental females were group-housed with age-matched females since weaning and were isolated in experimental observation cages (same as the males) for two nights (ca. 48 h) prior to the FIT. These females were also termed "residents", although in contrast to the males we do not know whether they consider the observation cage as their territory. The FIT started when a female intruder (unfamiliar to the test female and 10–30% less body weight – the differences in body weight between residents and intruders were always balanced over experimental groups) was placed into the observation cage. The duration of the test was 10 min, which has been shown to yield reliable information about individual aggression levels in the RI-test [64]. Behavior was videotaped, and ongoing behavior was continuously scored from video by an experienced observer blind to line/treatment using the JWatcher event-recorder program [65]. Similar to RI-tests, we scored the attack frequency and latency to first attack, as well as percentage of time displaying the following behaviors: attack, threat, lateral threat, offensive upright position, keeping down the intruder, and offensive grooming of the intruder (these six behaviors were pooled as "aggressive behavior"), social investigation (olfactory or tactile investigation of the intruder that was not aggressive, defensive, or sexual in nature), defensive behavior (submissive posture, kicking a following intruder with hind limb), sexual behavior (mounting, hopping, darting, lordosis), cage exploration, autogrooming, eating/drinking, and immobility (these last four behaviors were pooled as "non-social behavior"). Autogrooming was also separately analyzed as a potential marker of anxiety or OXT-induced stereotyped behavior [59,66], but no differences were found between any of the experimental groups and these data are not further discussed.

Elevated Plus-Maze

For analysis of anxiety-related behavior, resident females were tested on the elevated plus-maze (EPM) in the early dark phase (13.00–16.00 h) with lights on. The EPM consists of a plus-shaped platform elevated 80 cm above the floor, with two open (50×10 cm; 100 lux) and two closed arms (50×10×40 cm; 20 lux). Rats were placed in the center square facing a closed arm. The following parameters were recorded during the 5-min test using a video/computer system (Plus-maze version 2.0; Ernst Fricke): time spent in open and closed arms, number of entries into open and closed arms, latency to enter an open arm. Here, statistical analysis is only presented for the percentage of time spent on the open arms: [time on open arms]/[time on open+closed arms] × 100% as indication of anxiety levels.

Determination of Estrous Phase

In experiment 1, the estrous phases of residents and intruders were determined using vaginal smears that were taken with moistened cotton swabs, spread on object glasses, dipped in Nissl solution, and dehydrated in an alcohol series. The predominant presence of nucleated epithelial cells (proestrus), anucleated cornified epithelial cells (estrus), leukocytes (metestrus), or a low

number of mixed cells (diestrus) in the smears was assessed under a microscope [67]. Residents and intruders were subsequently pooled into two categories: receptive (proestrus/estrus) or non-receptive (metestrus/diestrus). Vaginal smears were taken after the FIT, to avoid stress-induced alterations in aggressive behavior [61].

Cannula Placement

For stereotaxic implantation of ICV guide cannulae, rats were anesthetized (isoflurane, Forene®, Abbott GmbH & Co. KG, Wiesbaden, Germany), injected i.p. with an antibiotic (3.0 mg/120 µl/animal Baytril®, Bayer Vital GmbH, Leverkusen, Germany), and mounted on a stereotaxic frame. An ICV guide cannula (21 G, 12 mm; Injecta GmbH, Germany) was implanted resting 2 mm above the lateral ventricle (1.0 mm caudal to bregma, 1.6 mm lateral to midline, 2.0 mm beneath the skull surface, [68]), fixed to the skull with two jeweller's screws and dental cement (Kallocryl, Speiko-Dr. Speier GmbH, Muenster, Germany), and closed by a stainless steel stylet (25 G). After surgery, each rat was allowed to recover for 48 h in her observation cage; stylets were cleaned on the day between surgery and the FIT.

ICV Infusion

Synthetic OXT (Sigma Aldrich Biochemicals, Steinheim, Germany) was infused at a dose of 0.1 µg/5 µl. This dose was carefully chosen to be low enough to avoid stereotyped barrel rotations (as often seen in female Wistar rats at 1.0 µg/5 µl, personal communication, M. Lukas), but high enough to reliably affect social behaviors in male rats [50]). For ICV infusion of either synthetic OXT or VEH (5 µl Ringer's solution, pH = 7.4, B. Braun AG, Melsungen, Germany), a 25 G infusion cannula extending 2 mm beyond the guide cannula and connected via polyethylene tubing to a Hamilton syringe was inserted into the guide cannula. After slow manual infusion the system was left in place for 10 s. Infusions took place 20 min prior to the FIT. After completion of the experiment, cannula placement was verified post mortem by ICV infusion of 2–3 µl black ink (Pelikan 4001, Hannover, Germany).

Immunohistochemistry

For immunohistochemical staining of pERK and OXT, rats were deeply anesthetized with isoflurane (Forane, Baxter, Deerfield, IL, USA) immediately after the end of the 10-min FIT and perfused transcardially with 0.1 M phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA) in an adjacent room. All 4% PFA perfusions started within 3 min after the end of the FIT. Fixated brains were dissected and immersed in 40 ml 4% PFA and post-fixated for 1 h before being stored in 0.1 M PBS at 4°C. Three to four days prior to cutting, brains were submerged in 30% phosphate buffered sucrose until saturated. The cryoprotected brains were snap-frozen on dry ice and cut into 40-µm thick sections on a cryostat set at −19°C. Consecutive sections were collected in six vials containing 5 ml 0.1 M PBS, to enable up to six different immunohistochemical stainings. Brain tissue was protected against mold with 50 µl 0.01% sodium azide per vial.

For single pERK staining, one series of brain sections (from a total of six) per animal was placed in a well on a 24-wells plate containing 3 ml of 0.1 M PBS. Brain sections from all individuals in the experiment were stained simultaneously. Staining started by washing the sections 3×10 min with fresh 0.1 M PBS, followed by 30 min of 0.1% H₂O₂ and another round of 3×10 min 0.1 M PBS. Tissue was then pre-incubated for 30 min with 0.1 M PBS containing 0.1% bovine serum albumin and 0.3% Triton-X-100

(PBS-BT) and incubated overnight at 4°C with PBS-BT containing rabbit-anti-pERK antibody (1:250, #4370, Cell Signaling, Danvers, MA, USA). The next day, tissue was washed 3×20 min with 0.1 M PBS. Brains were then incubated for 90 min with PBS-BT containing biotinylated goat-anti-rabbit antibody (1:200, BA-1000, Vector Laboratories, Inc., Burlingame, CA, USA), followed by 3×20 min washing with 0.1 M PBS. Staining was enhanced by 90 min of incubation with PBS-BT containing ABC-vector (1:800, PK-6100, Vector Laboratories, Inc., Burlingame, CA, USA), followed by 3×20 min washing with 0.1 M PBS. Tissue was then stained with a DAB-staining kit (SK-4100, Vector Laboratories, Inc., Burlingame, CA, USA) resulting in a blue-black cytoplasmic staining, and washed 3×15 min in 0.1 M PBS. Brain sections were mounted on adhesive microscope slides (Superfrost® Plus, Thermo Fisher Scientific Inc., Waltham, MA, USA), dehydrated in increasing ethanol concentrations, cleared and embedded in Roti®-Histol and Roti®-Histokitt (Carl Roth GmbH, Karslruhe, Germany), and coverslipped.

For pERK and OXT double staining, four brain sections containing the PVN were selected for each individual, and placed in a well (one per individual) on a 24-wells plate containing 1 ml of 0.1 M PBS. Again, brain sections from all individuals in the experiment were stained simultaneously. Staining started by washing the sections 3×10 min with fresh 0.1 M PBS, followed by 10 min incubation in ice-cold methanol at −20°C, 1 h of incubation in blocking solution (0.1 M PBS with 5% normal goat serum and 0.3% triton-x-100), and 64 h of incubation in antibody solution (0.1 M PBS with 1% bovine serum albumin and 0.3% triton-x-100) containing rabbit-anti-pERK antibody (1:250, Cell Signaling #4370, Danvers, MA, USA) and mouse-anti-OXT antibody (1:400, kind gift from Prof. Dr. Gainer, NIH, Bethesda, USA, 60). After incubation, brain sections were washed 3×10 min in 0.1 M PBS and (in the dark) incubated for 120 min with green fluorescent goat-anti-rabbit antibody (Dy-Light®488, 1:200, DI-1488, Vector Laboratories, Inc., Burlingame, CA, USA) followed by 3×10 min washing in 0.1 M PBS and 120 min incubation in red fluorescent goat-anti-mouse antibody (Dy-Light®594, 1:200, DI-2549, Vector Laboratories, Inc., Burlingame, CA, USA). Finally, brain sections were washed 3×10 min in 0.1 M PBS, mounted on adhesive microscope slides (Superfrost® Plus, Thermo Fisher Scientific Inc., Waltham, MA, USA) and embedded in Vectastain hard-set mounting medium (H-1400, Vector Laboratories, Inc., Burlingame, CA, USA). Slides were kept overnight in the dark at 4°C.

Immunohistochemical Analysis

Single labeling of pERK was analyzed in ten brain areas as derived from the Paxinos rat brain atlas [68]: the ventral part of the lateral septal nucleus (LSv), the medial division of the posteromedial bed nucleus of the stria terminalis (BSTmpm), the medial parvocellular and lateral magnocellular parts of the paraventricular hypothalamic nucleus (PVNmp and PVNlm), the hypothalamic attack area (HAA), the ventrolateral part of the ventromedial hypothalamic nucleus (VMHvl), the posterodorsal medial and central parts of the amydaloid nucleus (MeApd, CeA), the dorsal part of the dorsal raphe nucleus (DRd) and the ventrolateral periaqueductal gray (PAGvl). These brain areas were selected based on two criteria: a known role in aggressive behavior [15,27–31], and a considerable presence of pERK-IR in at least one of the two experimental groups. For each brain area, the optimal section was selected using a Leica DM5000B microscope connected to a personal computer; a photograph was taken at 10x magnification using Leica Application Suite v3.7 software. The number of pERK-IR neurons was counted in a 200×200 µm square placed over a representative area within the selected nucleus, using ImageJ software (NIH).

Double labeling of pERK and OXT was analyzed using the same microscope. For each individual the section with the highest density of OXT neurons in the PVNlm was selected. Images (one per animal, either left or right half of the selected PVNlm) were taken from the red and green channels without adjusting the focal plane in between, using L400W software installed on a personal computer connected to the microscope. Images were imported into Fiji (http://fiji.sc/wiki/index.php/Fiji), which is an open source image-processing package based on ImageJ software (National Institutes of Health). In each image the background was subtracted and the brightness and contrast adjusted so that cell bodies were highlighted and axons de-emphasized. Then, the two-channel readings for green and red fluorescence were merged and an area of 800×800 pixels containing the highest density of OXT neurons was defined. Within this area, single (OXT, pERK) and double (OXT+pERK) labeled neurons were counted; only neurons that demonstrated the same morphology, orientation and position in both red and green were considered doubly labeled.

Statistics

All data were analyzed by SPSS 19.0 software. As the assumption of normality was strongly violated for our most important behavioral parameters (i.e., skewed to the right for attack frequency and percentage of time spent behaving aggressively, skewed to the left for attack latency; Shapiro-Wilk tests: $p < 0.01$), behavioral data are presented as medians and quartiles and non-parametric tests were used to analyze behavior. Overall differences in behavior between multiple experimental groups were analyzed using Kruskal-Wallis tests, followed (when appropriate) by post-hoc pair-wise comparisons using Mann-Whitney tests for between-subjects analyses or Wilcoxon rank tests for within-subjects analyses (i.e., changes in behavior over time). Fisher's exact test (4×2 design) was used to analyze the proportion of females attacking in each of the four estrous cycle groups. Correlations between anxiety behavior in the EPM and aggressive behavior in the FIT were analyzed using the non-parametric Spearman's correlation coefficient (ρ). Neuronal activation in tolerant and aggressive females was compared using student's t-tests. All tests were performed two-sided and the level of significance was set at $p < 0.05$, but strong trends ($p < 0.07$) are reported and discussed as well.

Results

Exp. 1. Overall Behavior in the FIT: No Effect of Estrous Cycle (Fig. 2, Table 1)

The behavior of young female NAB rats in the FIT (n = 69) was analyzed and compared with the behavior of 108 adult male NAB rats in the RI-test. Due to the difference in age and housing conditions between females tested in the FIT and males tested in the RI-test, a statistical analysis was deemed inappropriate. The following comparison serves merely an illustrative purpose. Approximately 40% of the females (compared to 54% of males) attacked the intruder, with a marked variability in attack frequency (1 to 16 attacks for both females and males, Fig. 2A). Female residents spent 6.7% of the time displaying aggressive behavior (compared to 8.3% in males, Fig. 2B), and showed the same types of offensive behavior as male residents (Fig. 2C). Both female and male residents spent the largest proportion of the 10-min test displaying non-social behaviors (∼50–60%; exploration, auto-grooming, eating/drinking, Fig. 2B), but females spent more

time displaying non-aggressive social behavior, i.e. investigating the intruder, compared with males (~35% vs. 20%, Fig. 2B). Defensive and sexual behaviors were only occasionally observed in female rats (data not shown). Percentage of time spent behaving aggressively did not correlate with the difference in body weight between resident and intruder ($\rho = -0.132$, $p = 0.278$).

The four estrous cycle groups (receptive residents confronted with either receptive or non-receptive intruders; non-receptive residents confronted with either receptive or non-receptive intruders) did not differ in the proportion of residents that attacked the intruder (Fisher's exact test: $P = 0.43$), nor in percentage of aggressive, social and non-social behaviors displayed in the FIT (for statistical analysis, see Table 1).

Exp. 2. Effects of Innate Anxiety on Female Aggressive Behavior (Fig. 3, Table 2)

Adolescent (and adult, data not shown) HAB, NAB and LAB females differed significantly in the percentage of time spent on the open arms of the EPM (Fig. 3A). As expected, HAB females spent the least time in the open arms and LAB females the most. In the FIT, adolescent HAB, NAB and LAB rats differed significantly in attack latency (Fig. 3B) and attack frequency (data not shown), as well as percentage of time displaying aggressive behavior (Fig. 3C), social behavior (Fig. 3D) and non-social behavior (data not shown). Adolescent HAB females had shorter attack latencies, higher attack frequencies and spent more time displaying aggressive behavior than NAB and LAB females. In contrast, adult HAB, NAB and LAB rats did not differ in any aspect of aggression (see exp. 3). In addition, both adolescent and adult NAB females spent more time displaying non-aggressive social behavior than HAB and LAB females (for adolescents: see Table 2; for adults: $\chi^2(2) = 13.33$, $p = 0.001$; NAB vs LAB and HAB: $Z < -2.73$, $p < 0.01$). Lastly, adolescent but not adult LAB females spent more time displaying non-social behaviors compared to HAB and NAB females. For statistical analysis, see Table 2.

The level of anxiety correlated positively with the level of aggression (i.e., the percentage of time spent on the open arm of the EPM correlated *negatively* with the percentage of time spent behaving aggressively in the FIT) when adolescent females from all three selection lines were pooled ($\rho = 0.394$, $p = 0.006$, fig. 3E). However, no significant correlations between anxiety and aggres-

sion were found within the three selection lines (HAB: $\rho = 0.301$, NAB: $\rho = -0.072$, LAB: $\rho = 0.126$; $p > 0.342$).

Exp. 3. ICV OXT Inhibits Aggressive Behavior in Adult NAB and LAB Females (Fig. 4, Table 3)

Adult LAB and NAB females were found to be more aggressive in FIT 2 compared with FIT 1 when treated with VEH prior to FIT 2, whereas HAB rats showed no change after repeated testing. This increase in aggression seen in LAB and NAB rats was prevented by acute ICV OXT infusion prior to FIT 2. As a result, OXT-treated NAB and LAB females displayed significantly less aggressive behavior (percentage of time) compared with respective VEH groups. Infusion of OXT did not alter the percentage of social behavior compared with VEH in any of the rat lines. For statistical analysis see Table 3.

As a reference, 24 adult NAB females (10–11 weeks of age) were tested in the FIT twice, without surgical or any other intervention. 18 out of 24 females showed an increased percentage of aggressive behavior in FIT 2 compared with FIT 1 (Wilcoxon test for paired samples: $Z = -2.114$, $p = 0.034$; insert Fig. 4).

Exp. 4. Aggressive Behavior is Associated with Altered pERK-IR (Fig. 5 and 6)

Behavioral analysis of 23 adolescent NAB females resulted in two experimental groups ($n = 7$ each, 9 females were excluded for reasons explained above) that differed significantly in the median percentage of aggressive behavior (non-aggressive females: 4.5% (quartiles: 3.8–5.7%); aggressive females: 24.3% (22.5–26.6%); $Z = -3.13$, $p < 0.01$) and percentage of non-social behaviors (non-aggressive females: 45.2% (44.9–51.2%); aggressive females: 39.8% (30.1–41.2%), $Z = -2.62$, $p < 0.01$). Both groups showed a similar amount of non-aggressive social behaviors ($Z = -1.09$, $p = 0.28$).

Females that had been aggressive in the FIT showed a lower level of pERK-IR in the PVNml ($t = -2.81$, $p < 0.05$) and increased pERK-IR in the HAA ($t = 2.41$, $p < 0.05$) compared with non-aggressive females (Fig. 5). Interestingly, receptive residents showed reduced pERK-IR in the BSTmpm compared to non-receptive residents (14.6 ± 1.9 vs. 8.1 ± 1.6, $p < 0.05$, data not shown), independent of the amount of aggressive behavior.

Double labeling immunofluorescence for OXT and pERK in the PVNlm (Fig. 6) revealed that aggressive females showed a
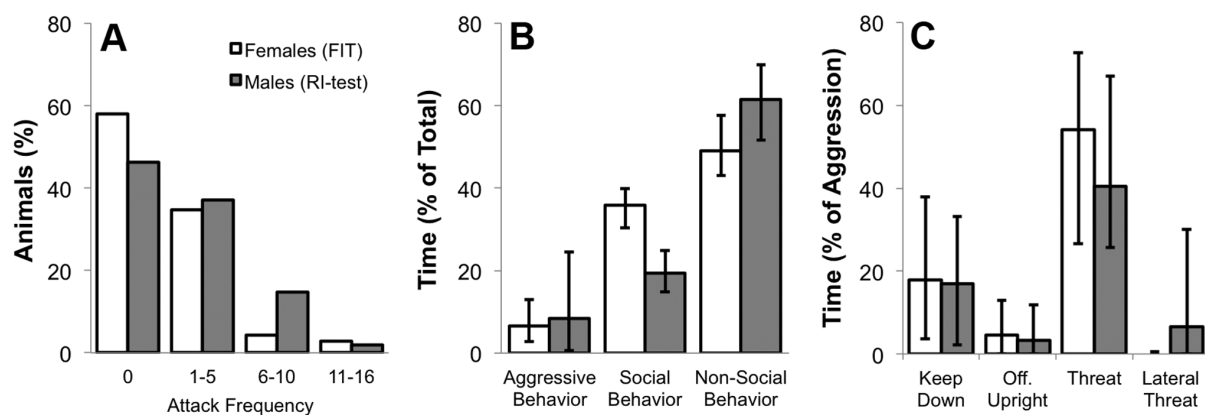

Figure 2. Behavioral profile of adult (10–11 week old) virgin female NAB residents (n = 69) during their first 10-min female intruder test (FIT), and adult (16–22 week old) male NAB residents (n = 108) during their first 10-min resident-intruder test (RI-test). A: attack frequency distribution; **B:** aggressive, social and non-social behavior as percentage of total behavior; **C:** different types of offensive behavior as percentage of total aggressive behavior. Data are medians and quartiles. A statistical comparison of the sexes was not performed.
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Table 1. Effect of estrous phase on behavior in the FIT.

Phase Resident:	Receptive		Non-Receptive		$\chi^2(3)$; P
Phase Intruder:	Rec.	Non-Rec.	Rec.	Non-Rec.	
N (total = 69):	15	20	11	23	
Fem. Attacking	5 (33%)	11 (55%)	3 (27%)	10 (43%)	[Fisher: P = 0.43]
Aggressive Behavior	8.90; 3.9−12.5	9.24; 5.1−12.5	3.82; 2.2−6.42	6.19; 0.7−18.0	4.13; 0.25
Social Behavior	35.32; 30.6−39.1	32.72; 28.8−41.4	38.83; 36.6−41.5	34.72; 31.7−38.1	3.73; 0.29
Non-Social Behavior	51.60; 43.0−60.0	46.39; 41.4−55.0	49.76; 48.3−55.5	48.79; 45.0−57.5	1.33; 0.72

The number of females attacking as well as the percentage aggressive, social and non-social behavior of receptive and non-receptive adult (10–11 week old) virgin female NAB residents in response to receptive and non-receptive virgin female intruder rats in a 10-min Female Intruder Test (FIT). Data are medians and quartiles.
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reduced number of double-labeled neurons (t = −2.37, p<0.05) and, likewise, a reduced percentage of OXT neurons that showed pERK-IR (t = −2.72, p<0.05).

Discussion

The experiments reported here confirm that aggression is an important component of the behavioral repertoire of adolescent and adult virgin female rats, and can be reliably quantified in the FIT, i.e. during confrontation with a smaller female intruder rat following 48 h of isolation. Further, female aggression seems to be independent of the estrous cycle of both residents and intruders, but is correlated with the innate level of anxiety-related behavior

with highest aggression levels found in adolescent HAB females. Female aggression is associated with reduced neuronal activity in the hypothalamic PVN, specifically in local OXT neurons, and central infusion of OXT results in reduced aggression in the FIT. These results indicate an important involvement of the brain OXT system in female aggression regulation.

We compared behavior of female residents in the FIT with behavior of male residents in the RI-test (based on recent results, see [63]). Although the differences between the paradigms (housing conditions, age of testing) preclude a direct statistical comparison of the sexes, a descriptive comparison of the behavior of male and female residents during their first exposure to the FIT
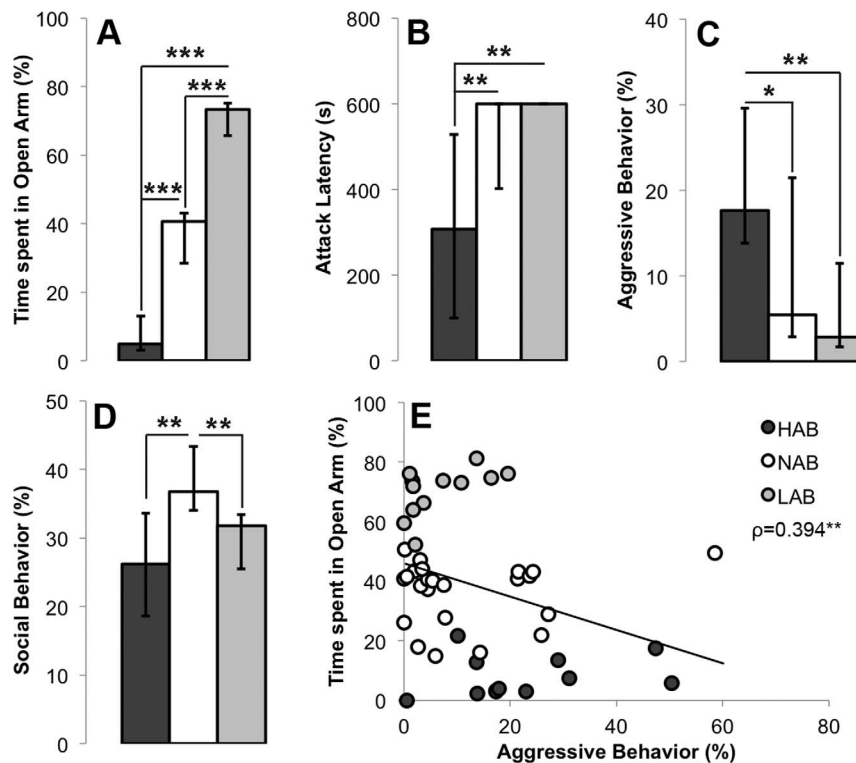

Figure 3. Behavioral profile of adolescent (7–8 week old) virgin female HAB (n = 12), NAB (n = 23) and LAB (n = 12) rats in the EPM and FIT. A: Percentage of time spent in the open arms of the EPM; B–D: Attack latency, percentage of time displaying aggressive behavior and percentage of time displaying social behavior in a 10-min FIT. Data in A–D are medians and quartiles. E: Spearman's correlation coefficient between percentage of time spent in the open arms of the EPM and percentage of time spent displaying aggressive behavior in the FIT two days later. *P< 0.05; **P<0.01; ***P<0.001.
doi:10.1371/journal.pone.0091701.g003

Table 2. Effect of innate anxiety levels on behavior in the FIT.

Behavior	Test	HAB/NAB/LAB $\chi^2(2)$; P	Pair-wise Comparison	Z
Time in Open Arm (%)	EPM	38.18; <0.001	HAB vs. NAB	−4.62***
			HAB vs. LAB	−4.18***
			NAB vs. LAB	4.80***
Attack Latency (s)	FIT	11.82; <0.01	HAB vs. NAB	−2.78**
			HAB vs. LAB	−2.96**
			NAB vs. LAB	− − −
Attack Frequency (n)	FIT	13.52; <0.01	HAB vs. NAB	−2.91**
			HAB vs. LAB	−3.21**
			NAB vs. LAB	− − −
Social Behavior (%)	FIT	13.83; <0.01	HAB vs. NAB	−3.27**
			HAB vs. LAB	−2.64**
			NAB vs. LAB	− − −
Aggressive Behavior (%)	FIT	10.32; <0.01	HAB vs. NAB	−2.26*
			HAB vs. LAB	−3.41**
			NAB vs. LAB	− − −
Non-social Behavior (%)	FIT	6.58; <0.05	HAB vs. NAB	− − −
			HAB vs. LAB	−2.31*
			NAB vs. LAB	−2.22*

Overall effects (Kruskal-Wallis) and Mann-Whitney pair-wise comparisons for experiment 2 (Fig. 3).
***P<0.001,
**P<0.01,
*P<0.05.
doi:10.1371/journal.pone.0091701.t002

or RI-test can be made. Overall, female residents showed a somewhat lower level of aggression compared with males (42% vs. 54% attacked their intruder at least once, median time spent displaying aggression was 6.7% vs. 8.3%). These differences between the sexes were much smaller than expected, however, a direct comparison of males and females of the same age in the same paradigm is needed to draw any definite conclusions. Nevertheless, combined with the recent report of considerable aggression in female Wistar rats in a different paradigm [47], these data point in the direction of a persistent under-estimation in the literature of female aggression in rats.

It should be noted here that the 48 h isolation period in females was, in contrast to the 10 days of pair-housing in males, not intended to instigate aggression, but rather to allow recovery from stereotactic surgeries needed for (for example) neuropharmacological intervention. In order to enable comparisons between various experiments, we standardized the isolation period at least in this set of studies. However, significantly shorter or longer bouts of isolation, as well as socially instable housing paradigms [69], are expected to alter general social and aggressive interactions, which would need to be studied in more detail.

Our finding of a similar median percentage of time being aggressive of non-receptive and receptive residents, independent of
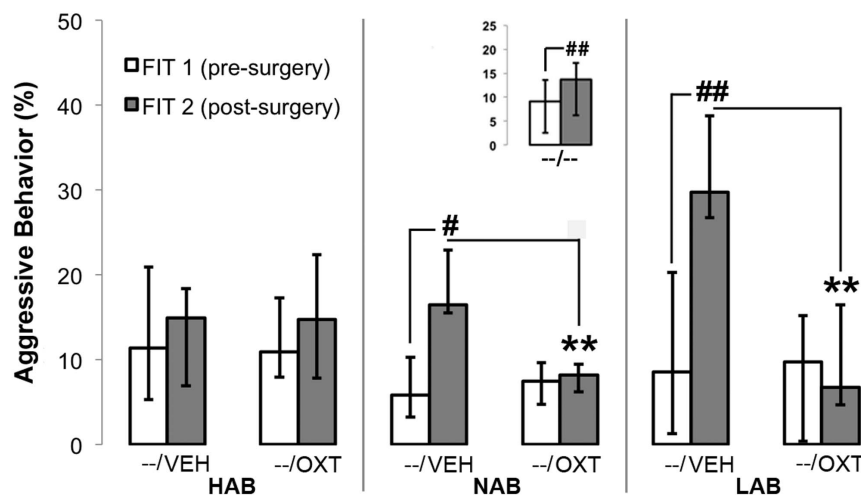

Figure 4. Percentage of aggressive behavior of adult (10–11 week old) virgin female HAB, NAB and LAB rats in two 10-min FITs, five days prior to surgery (FIT 1) or two days after surgery (FIT 2). 20 min prior to FIT 2, rats were ICV infused with either VEH (5 µl Ringer, n = 10 per selection line) or OXT (0.1 µg/5 µl Ringer, n = 10 per selection line). Insert shows data for unmanipulated NAB rats (no surgery performed, n = 24). **Significant difference with corresponding VEH data (p<0.01). #/## Significant difference with corresponding FIT 1 data (# p = 0.06, ## P<0.05).
doi:10.1371/journal.pone.0091701.g004

Table 3. Effect of ICV infusion of OXT on behavior in the FIT.

Comparison:		OXT vs VEH	FIT 2 vs FIT 1	
Behavior	Rat Line	Z	Z [VEH]	Z [OXT]
Aggressive Behavior (%)	HAB	− − − −	− − − −	− − − −
	NAB	−3.02**	−1.89#	− − − −
	LAB	−2.95**	−2.60**	− − − −
Attack Latency (s)	HAB	− − − −	− − − −	− − − −
	NAB	− − − −	− − − −	− − − −
	LAB	− − − −	−2.29*	− − − −
Social Behavior (%)	HAB	− − − −	− − − −	− − − −
	NAB	− − − −	− − − −	− − − −
	LAB	− − − −	−2.10*	− − − −
Non-social Behavior (%)	HAB	− − − −	− − − −	− − − −
	NAB	−1.89#	− − − −	− − − −
	LAB	−2.04*	− − − −	− − − −

Mann-Whitney pair-wise comparisons for experiment 3 (Fig. 4).
**$P<0.01$,
*$P<0.05$;
#$P=0.06$.
doi:10.1371/journal.pone.0091701.t003

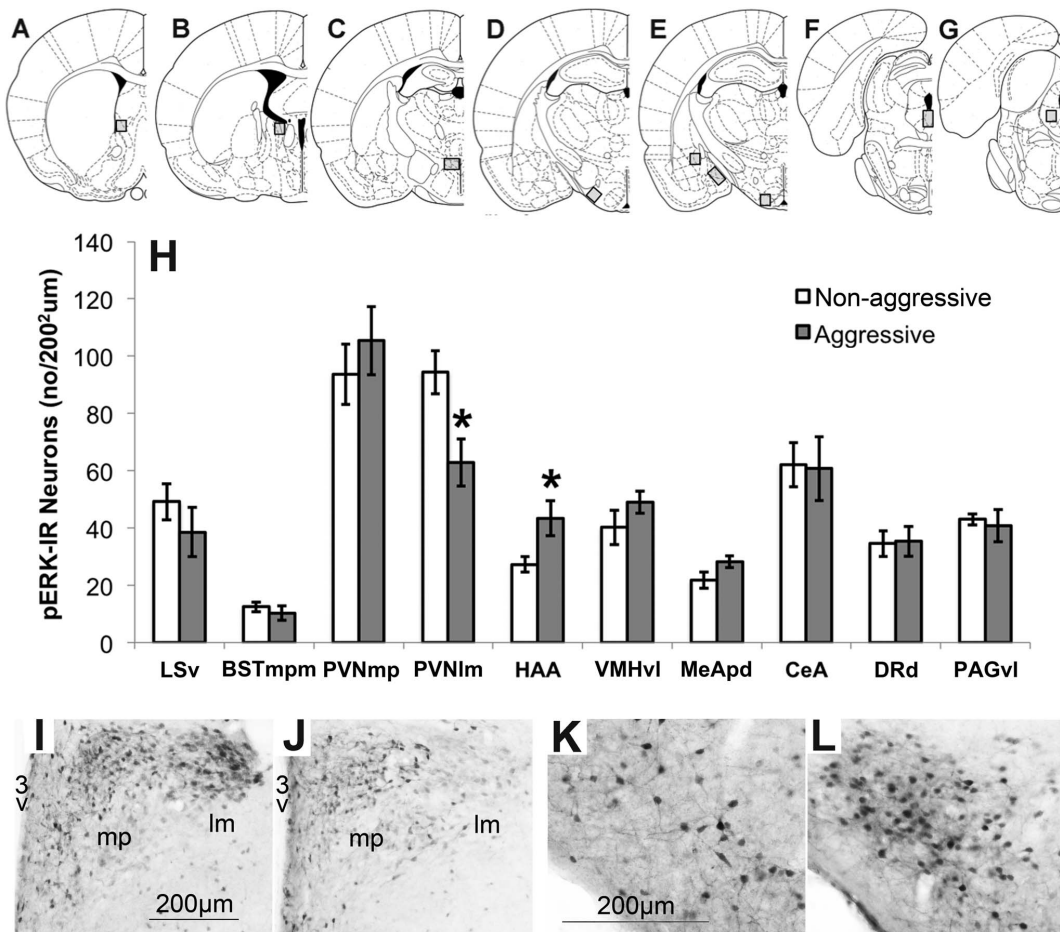**Figure 5. Immunohistochemical single-labeling of pERK. A–G:** schematic drawings of coronal brain sections (derived from the Paxinos rat brain atlas, 63) with grey squares corresponding to the location of the quantified brain areas (**A:** LSv, bregma +0.20; **B:** BSTmpm, bregma −0.80; **C:** PVNm and PVNl, bregma −1.80; **D:** HAA, bregma −2.80; **E:** MeApd, CeA, VHMvl, bregma −3.14; **F:** DRd, bregma −7.80; **G:** PAGvl, bregma −8.30; **H:** Average density of pERK-IR in non-aggressive (white bars, n = 7) compared with aggressive (grey bars, n = 7) adolescent (7–8 week old) female NAB rats. Data are means ± s.e.m; *$P<0.05$; **I–J:** Illustration of pERK-IR in the PVNmp (''mp'') and PVNlm (''lm'') of non-aggressive (left) and aggressive (right) individuals (3v = third ventricle); **K–L:** Illustration of pERK-IR in the HAA of non-aggressive (left) and aggressive (right) individuals.
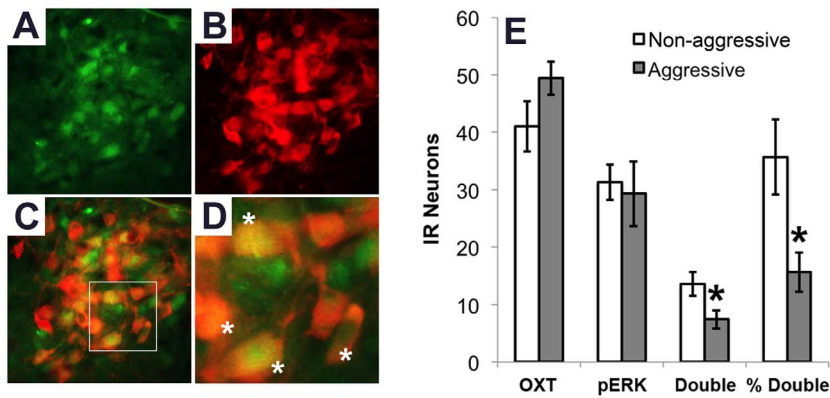doi:10.1371/journal.pone.0091701.g005

Figure 6. Immunohistochemical double-labeling of OXT and pERK. A–C: Illustrations of OXT (green) and pERK (red) IF in the PVNlm of a non-aggressive female. Total area of A, B and C corresponds to the size of the analyzed area; **D:** Higher magnification of the white square in C, white asterisks signify double-labeled neurons; **E:** Density of single- and double-labeled neurons as well as the percentage of OXT-IR neurons that also showed pERK-IR in non-aggressive (white bars, n = 7) and aggressive (grey bars, n = 7) adolescent female NAB rats. Data are means ± s.e.m; *P<0.05. doi:10.1371/journal.pone.0091701.g006

the estrous cycle of the intruder, is consistent with results reported by Cordero et al. [47]. However, Ho et al. [46] reported reduced aggression in receptive adult female Wistar rats. The difference in findings can be explained by the experimental conditions in the latter study, in which the females were sexually experienced, had been pseudo-pregnant, were isolated for at least four weeks prior to the experiment and were confronted with same-size, ovariec-tomized (i.e., non-receptive) intruder females. These experiences are likely to have significantly altered their behavior toward an intruder. Nevertheless, determining the estrous phase of the residents and intruders following the FIT remains advisable to confirm our findings under different experimental conditions or in other rat strains.

High levels of innate anxiety were associated with increased aggression in adolescent females, but this association disappeared in adulthood. In adolescence as well as in adulthood, HAB (and LAB) females displayed less social behavior toward their intruder than NAB females. These findings are consistent with other results from our lab showing altered social behavior in HAB females. For example, 5-week old female HAB juveniles display significantly reduced play-fighting behavior accompanied by reduced ultrason-ic vocalizations compared with NAB and LAB juveniles (M. Lukas, personal communication). In addition, lactating HAB females show increased levels of maternal aggression compared to LAB and NAB dams [35]. In humans, anxiety disorders (especially social phobia) are often co-morbid with behavioral problems including aggression, especially during adolescence [70,71], and this link appears to be even stronger in girls compared to boys [72,73]. Taken together, juvenile and adolescent HAB females may form a promising animal model to further explore the link between high anxiety and aggression.

ICV infusion of synthetic OXT inhibited aggression in both NAB and LAB females. The demonstration of an anti-aggressive effect of OXT in virgin female rats is in agreement with recent findings in adult male wild-type Groningen rats [59]. However, our effects were found at a 10-fold lower dose (0.1 versus 1 µg/5 µl), potentially because female rats are more sensitive to the anti-aggressive effects of OXT. Our findings are also in concordance with the results of Harmon et al. [58], who showed inhibition of aggression by OXT in virgin female Syrian hamsters. The capacity of central OXT to reduce aggression in virgin females has to be clearly separated from its substantial promotion of maternal aggression in lactating animals. Maternal aggression is

part of the complex patterns of maternal behavior to protect the offspring [42], and OXT was shown to facilitate the defensive behavior of lactating dams specifically within the PVN [35] and the amygdala [74]. Future research is needed to further determine the extent to which the neuronal circuitries involved in female aggression of virgin rats and of lactating dams differ.

In the present experiment, HAB females did not reduce their aggressive behavior in response to OXT infusion, in contrast to NAB and LAB females. This suggests a difference in the neurobiological underpinnings of aggressive behavior in the different rat lines. Indeed, HAB rats are known to overexpress AVP due to impaired repression at a AVP promotor polymor-phism [75]. Increased AVP exerts a strong control over aggression in males [18,76], and lactating HAB females show increased release of AVP in the central amygdala during maternal defense [77]. Future research is needed to confirm a role of AVP in aggressive adolescent female HAB rats.

Vehicle-treated LAB rats and, to a lesser extent, NAB rats showed increased aggression when exposed to the FIT a second time. Surgery and subsequent vehicle-infusion resulting in increased levels of stress and anxiety may have contributed to the increased aggression. However, a similar increase was seen in non-manipulated NAB females tested twice in the FIT without surgery in between. These findings are consistent with multiple reports of increased aggression induced by fighting experience in male rats and house mice, and in male and female California mice and Syrian hamsters [61,78–82].

To this date virtually nothing is known about the neurobiolog-ical underpinnings of non-maternal female aggressive behavior, especially in the gregarious rat. Therefore, we analyzed local patterns of neuronal activation (density of pERK-IR neurons) in aggressive and non-aggressive female residents [61,62]. Interest-ingly, aggressive females had a lower number of pERK-IR neurons in the PVNlm compared to non-aggressive females. Since this difference in pERK-IR was found at the approximate location of OXT neurons, double labeling immunofluorescence was performed confirming that aggressive females had reduced pERK-IR in OXT neurons compared to tolerant females. This finding is consistent with results from female California mice housed under shortened day lengths that showed both increased aggressive behavior and reduced immunoreactivity of pERK and oxytocin in the PVN, as well as reduced OXT release in the blood following an RI-test [62]. Although these results do not

demonstrate a causal link, they imply that the increased level of pERK-IR in OXT neurons in non-aggressive females is related to increased OXT release within the PVN and/or central target regions, the more as the ERK1/2 pathway is functionally able to alter the firing of OXT neurons [83]. Combined with the anti-aggressive actions of central OXT infusion in female rats, we postulate that the level of aggressive behavior toward an intruder displayed by a female rat depends at least in part on the activity of the brain OXT system and the central release of OXT. A similar pathway may be present in male rats [59,84].

Aggressive females showed a higher density of pERK-IR neurons in the HAA, which is consistent with the increased neuronal activation in this area in aggressive (male or lactating female) rats and mice [30,37,85,86] as well as with the well-known pro-aggressive function of this brain area demonstrated in male rodents [87]. Substantial pERK-IR was found in LSv, BSTmpm, PVNmp, VMHvl, CeA, MeApd, DRd and PAGvl in FIT-exposed females, but no differences existed between non-aggressive and aggressive individuals. Further research is needed to study the neurobiological pathways and neuronal activation underlying female aggression in more detail, for example using different markers of neuronal activation such as Fos or Egr-1 (zif268).

In conclusion, aggressive behavior in virgin female Wistar rats can be monitored using the FIT – a simple modification of the RI-test established for studying inter-male aggression. Our findings show a strong inhibiting effect of OXT on female aggression, and the association between low brain OXT system activity and high female aggression. Future studies are needed to localize the effects of OXT, to show the involvement of the endogenous OXT system, and to investigate the moderating roles of AVP, 5-HT and steroid hormones (which are known to influence inter-male aggression) on female aggression. Together, these data will shed light on the, putatively sex-specific, neurobiological control of aggressive behavior in young girls and women.

Acknowledgments

Author Contributions

Conceived and designed the experiments: TRJ IDN. Performed the experiments: TRJ DIB. Analyzed the data: TRJ. Contributed reagents/materials/analysis tools: TRJ IDN. Wrote the paper: TRJ.

References

1. Archer J (2009) Does sexual selection explain human sex differences in aggression? Behav Brain Sci 32: 249–266.
2. Campbell A (1999) Staying alive: evolution, culture, and women's intrasexual aggression. Behav Brain Sci 22: 203–214; discussion 214–252.
3. Berkout OV, Young JN, Gross AM (2011) Mean girls and bad boys: Recent research on gender differences in conduct disorder. Aggress Violent Behav 16: 503–511.
4. Morcillo C, Duarte CS, Sala R, Wang S, Lejuez CW, et al. (2012) Conduct disorder and adult psychiatric diagnoses: Associations and gender differences in the U.S. adult population. J Psychiatr Res 46: 323–330.
5. Thornton AJV, Graham-Kevan N, Archer J (2012) Prevalence of women's violent and nonviolent offending behavior: A comparison of self-reports, victims' reports, and third-party reports. J Interpers Violence 27: 1399–1427.
6. Fairchild G, Hagan CC, Walsh ND, Passamonti L, Calder AJ, et al. (2013) Brain structure abnormalities in adolescent girls with conduct disorder. J Child Psychol Psychiatry 54: 86–95.
7. Collishaw S, Gardner F, Maughan B, Scot J, Pickles A (2012) Do historical changes in parent-child relationships explain increases in youth conduct problems? J Abnorm Child Psychol Psychol 40: 119–132.
8. Pajer KA (1998) What happens to "bad" girls? A review of the adult outcomes of antisocial adolescent girls. Am J Psychiatry 155: 862–870.
9. Lanctôt N, Cernkovich SA, Giordano PC (2007) Delinquent behavior, official delinquency, and gender: consequences for adulthood functioning and well-being. Criminology 45: 131–157.
10. Fontaine N, Carbonneau R, Barker ED, Vitaro F, Hébert M, Côté SM, Nagin D, Zoccolillo M, Tremblay RE (2008) Girls' hyperactivity and physical aggression during childhood and adjustment problems in early adulthood. Arch Gen Psychiatry 65: 320–328.
11. Odgers CL, Moffitt TE, Broadbent JM, Dickson N, Hancox RJ, et al. (2008) Female and male antisocial trajectories: from childhood origins to adult outcomes. Dev Psychopathol 20: 673–716.
12. Haller J, Kruk MR (2006) Normal and abnormal aggression: human disorders and novel laboratory models. Neurosci Biobehav Rev 30: 292–303.
13. Takahashi A, Quadros IM, Almeida RMM de, Miczek KA (2011) Brain serotonin receptors and transporters: initiation vs. termination of escalated aggression. Psychopharmacology (Berl) 213: 183–212.
14. Veenema AH, Neumann ID (2007) Neurobiological mechanisms of aggression and stress coping: a comparative study in mouse and rat selection lines. Brain Behav Evol 70: 274–285.
15. Nelson RJ, Trainor BC (2007) Neural mechanisms of aggression. Nat Rev Neurosci 8: 536–546.
16. Ferris CF (2000) Adolescent stress and neural plasticity in hamsters: a vasopressin-serotonin model of inappropriate aggressive behaviour. Exp Physiol 85: 85s–90s.
17. Veenema AH, Neumann ID (2008) Central vasopressin and oxytocin release: regulation of complex social behaviours. Prog Brain Res 170: 261–276 [eds. ID Neumann and R Landgraf].
18. Beiderbeck DI, Neumann ID, Veenema AH (2007) Differences in intermale aggression are accompanied by opposite vasopressin release patterns within the septum in rats bred for low and high anxiety. Eur J Neurosci 26: 3597–3605.
19. De Vries GJ (2008) Sex differences in vasopressin and oxytocin innervation of the brain. Prog Brain Res 170: 7–27 [eds. ID Neumann and R Landgraf].
20. Hines M (2011) Gender development and the human brain. Annu Rev Neurosci 34: 69–88.
21. Jovanovic H, Lundberg J, Karlsson P, Cerin A, Saijo T, et al. (2008) Sex differences in the serotonin 1A receptor and serotonin transporter binding in the human brain measured by PET. NeuroImage 39: 1408–1419.
22. Nishizawa S, Benkelfat C, Young SN, Leyton M, Mzengeza S, et al. (1997) Differences between males and females in rates of serotonin synthesis in human brain. Proc Natl Acad Sci U S A 94: 5308–5313.
23. Näslund J, Studer E, Nilsson K, Westberg L, Eriksson E (2013) Serotonin depletion counteracts sex differences in anxiety-related behaviour in rat. Psychopharmacology (Berl) 230: 29–35.
24. Joppa MA, Rowe RK, Meisel RL (1997) Effects of serotonin 1A or 1B receptor agonists on social aggression in male and female Syrian hamsters. Pharmacol Biochem Behav 58: 349–353.
25. Nephew BC, Bridges RS (2008) Central actions of arginine vasopressin and a V1a receptor antagonist on maternal aggression, maternal behavior, and grooming in lactating rats. Pharmacol Biochem Behav 91: 77–83.
26. Gutzler SJ, Karom M, Erwin WD, Albers HE (2010) Arginine-vasopressin and the regulation of aggression in female Syrian hamsters (Mesocritus auratus). Eur J Neurosci 31: 1655–1663.
27. Kollack-Walker S, Newman SW (1995) Mating and agonistic behavior produce different patterns of Fos immunolabeling in the male Syrian hamster brain. Neuroscience 66: 721–736.
28. Lin D, Boyle MP, Dollar P, Lee H, Lein ES, et al. (2011) Functional identification of an aggression locus in the mouse hypothalamus. Nature 470: 221–226.
29. Veening JG, Coolen LM, de Jong TR, Joosten HW, de Boer SF, et al. (2005) Do similar neural systems subserve aggressive and sexual behaviour in male rats? Insights from c-Fos and pharmacological studies. Eur J Pharmacol 526: 226–239.
30. Haller J, Tóth M, Halasz J, De Boer SF (2006) Patterns of violent aggression-induced brain c-fos expression in male mice selected for aggressiveness. Physiol Behav 88: 173–182.
31. Kruk MR (1991) Ethology and pharmacology of hypothalamic aggression in the rat. Neurosci Biobehav Rev 15: 527–538.
32. McCarthy MM, Arnold AP (2011) Reframing sexual differentiation of the brain. Nat Neurosci 14: 677–683.
33. Yang CF, Chiang MC, Gray DC, Prabhakaran M, Alvarado M, et al. (2013) Sexually dimorphic neurons in the ventromedial hypothalamus govern mating and aggression in males. Cell 153: 896–909.
34. Lonstein JS, Gammie SC (2002) Sensory, hormonal, and neural control of maternal aggression in laboratory rodents. Neurosci Biobehav Rev 26: 869–888.

35. Bosch OJ, Meddle SL, Beiderbeck DI, Douglas AJ, Neumann ID (2005) Brain oxytocin correlates with maternal aggression: Link to anxiety. J Neurosci 25: 6807–6815.

36. Olivier B, Mos J, van Oorschot R, Hen R (1995) Serotonin receptors and animal models of aggressive behavior. Pharmacopsychiatry 28 Suppl 2: 80–90.

37. Hasen NS, Gammie SC (2005) Differential fos activation in virgin and lactating mice in response to an intruder. Physiol Behav 84: 681–695.

38. Gammie SC, Nelson RJ (2001) cFOS and pCREB activation and maternal aggression in mice. Brain Res 898: 232–241.

39. Trainor BC, Crean KK, Fry WHD, Sweeney C (2010) Activation of extracellular signal-regulated kinases in social behavior circuits during resident-intruder aggression tests. Neuroscience 165: 325–336.

40. Davis E., Marler C. (2004) C-FOS changes following an aggressive encounter in female California mice: A synthesis of behavior, hormone changes and neural activity. Neuroscience 127: 611–624.

41. Joppa MA, Meisel RL, Garber MA (1995) c-Fos expression in female hamster brain following sexual and aggressive behaviors. Neuroscience 68: 783–792.

42. Bosch OJ, Neumann ID (2012) Both oxytocin and vasopressin are mediators of maternal care and aggression in rodents: From central release to sites of action. Horm Behav 61: 293–303.

43. Goodson JL, Kabelik D, Schrock SE (2009) Dynamic neuromodulation of aggression by vasotocin: influence of social context and social phenotype in territorial songbirds. Biol Lett 5: 554–556.

44. Karriker-Jaffe KJ, Foshee VA, Ennett ST, Suchindran C (2008) The development of aggression during adolescence: Sex differences in trajectories of physical and social aggression among youth in rural areas. J Abnorm Child Psychol 36: 1227–1236.

45. Albonetti ME, Farabollini F (1993) Restraint increases both aggression and defence in female rats tested against same-sex unfamiliar conspecifics. Aggress Behav 19: 369–376.

46. Ho HP, Olsson M, Westberg L, Melke J, Eriksson E (2001) The serotonin reuptake inhibitor fluoxetine reduces sex steroid-related aggression in female rats: an animal model of premenstrual irritability? Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol 24: 502–510.

47. Cordero MI, Ansermet F, Sandi C (2013) Long-term programming of enhanced aggression by peripuberty stress in female rats. Psychoneuroendocrinology 38: 2758–2769.

48. Henniger MSH, Ohl F, Hölter SM, Weißenbacher P, Toschi N, Lörscher P, Wigger A, Spanagel R, Landgraf R (2000) Unconditioned anxiety and social behavior in two rat lines selectively bred for high and low anxiety-related behavior. Behav Brain Res 111: 153–163.

49. Neumann ID, Veenema AH, Beiderbeck DI (2010) Aggression and anxiety: social context and neurobiological links. Front Behav Neurosci 4: 12.

50. Lukas M, Toth I, Veenema AH, Neumann ID (2013) Oxytocin mediates rodent social memory within the lateral septum and the medial amygdala depending on the relevance of the social stimulus: Male juvenile versus female adult conspecifics. Psychoneuroendocrinology 38: 916–926.

51. Lukas M, Toth I, Reber SO, Slattery DA, Veenema AH, et al. (2011) The neuropeptide oxytocin facilitates pro-social behavior and prevents social avoidance in rats and mice. Neuropsychopharmacology 36: 2159–2168.

52. Young LJ, Wang Z (2004) The neurobiology of pair bonding. Nat Neurosci 7: 1048–1054.

53. Pedersen CA, Boccia ML (2002) Oxytocin links mothering received, mothering bestowed and adult stress responses. Stress 5: 259–267.

54. Rimmele U, Hediger K, Heinrichs M, Klaver P (2009) Oxytocin makes a face in memory familiar. J Neurosci 29: 38–42.

55. Baumgartner T, Heinrichs M, Vonlanthen A, Fischbacher U, Fehr E (2008) Oxytocin shapes the neural circuitry of trust and trust adaptation in humans. Neuron 58: 639–650.

56. Bartz JA, Zaki J, Bolger N, Hollander E, Ludwig NN, et al. (2010) Oxytocin selectively improves empathic accuracy. Psychol Sci 21: 1426–1428.

57. Hurlemann R, Patin A, Onur OA, Cohen MX, Baumgartner T, et al. (2010) Oxytocin enhances amygdala-dependent, socially reinforced learning and emotional empathy in humans. J Neurosci 30: 4999–5007.

58. Harmon AC, Huhman KL, Moore TO, Albers HE (2002) Oxytocin inhibits aggression in female Syrian hamsters. J Neuroendocrinol 14: 963–969.

59. Calcagnoli F, Boer SF de, Althaus M, Boer JA den, Koolhaas JM (2013) Antiaggressive activity of central oxytocin in male rats. Psychopharmacology (Berl) 229: 639–651.

60. Campbell A, Hausmann M (2013) Effects of oxytocin on women's aggression depend on state anxiety. Aggress Behav 39: 316–322.

61. Silva AL, Fry WHD, Sweeney C, Trainor BC (2010) Effects of photoperiod and experience on aggressive behavior in female California mice. Behav Brain Res 208: 528–534.

62. Trainor BC, Takahashi EY, Silva AL, Crean KK, Hostetler C (2010) Sex differences in hormonal responses to social conflict in the monogamous California mouse. Horm Behav 58: 506–512.

63. Beiderbeck DI, Reber SO, Havasi A, Bredewold R, Veenema AH, et al. (2012) High and abnormal forms of aggression in rats with extremes in trait anxiety – Involvement of the dopamine system in the nucleus accumbens. Psychoneuroendocrinology 37: 1969–1980.

64. Koolhaas JM, Coppens CM, de Boer SF, Buwalda B, Meerlo P, et al. (2013) The Resident-intruder Paradigm: a standardized test for aggression, violence and social stress. J Vis Exp. Available: http://www.jove.com/video/4367/the-resident-intruder-paradigm-standardized-test-for-aggression.

65. Blumstein DT, Daniel JC (2007) Quantifying behavior the JWatcher way. Sinauer Associates, Inc., Sunderland, MA, USA.

66. Kalueff AV, Tuohimaa P (2005) The grooming analysis algorithm discriminates between different levels of anxiety in rats: potential utility for neurobehavioral stress research. J Neurosci Methods 143: 169–177.

67. Marcondes FK, Bianchi FJ, Tanno AP (2002) Determination of the estrous cycle phases of rats: some helpful considerations. Braz J Biol Rev Brasileira Biol 62: 609–614.

68. Paxinos G, Watson C (1997) The rat brain in stereotactic coordinates, compact 3rd ed. Academic Press, San Diego, CA, USA.

69. Baranyi J, Bakos N, Haller J (2005) Social instability in female rats: The relationship between stress-related and anxiety-like consequences. Physiol Behav 31: 511–518.

70. Hodgins S, Barbareschi G, Larsson A (2011) Adolescents with conduct disorder: does anxiety make a difference? J Forensic Psychiatry Psychol 22: 669–691.

71. Polier GG, Vloet TD, Herpertz-Dahlmann B, Laurens KR, Hodgins S (2012) Comorbidity of conduct disorder symptoms and internalising problems in children: investigating a community and a clinical sample. Eur Child Adolesc Psychiatry 21: 31–38.

72. Wasserman GA, McReynolds LS, Ko SJ, Katz LM, Carpenter JR (2005) Gender Differences in Psychiatric Disorders at Juvenile Probation Intake. Am J Public Health 95: 131–137.

73. Lehto-Salo P, Närhi V, Ahonen T, Marttunen M (2009) Psychiatric comorbidity more common among adolescent females with CD/ODD than among males. Nord J Psychiatry 63: 308–315.

74. Ferris CF, Foote KB, Meltser HM, Plenby MG, Smith KL, et al. (1992) Oxytocin in the amygdala facilitates maternal aggression. Ann N Y Acad Sci 652: 456–457.

75. Murgatroyd C, Wigger A, Frank E, Singewald N, Bunck M, et al. (2004) Impaired repression at a vasopressin promoter polymorphism underlies overexpression of vasopressin in a rat model of trait anxiety. J Neurosci Off J Soc Neurosci 24: 7762–7770.

76. Caldwell HK, Lee H-J, Macbeth AH, Young WS 3rd (2008) Vasopressin: behavioral roles of an "original" neuropeptide. Prog Neurobiol 84: 1–24.

77. Bosch OJ, Neumann ID (2010) Vasopressin released within the central amygdala promotes maternal aggression. Eur J Neurosci 31: 883–891.

78. Boer SF de, Vegt BJ van der, Koolhaas JM (2003) Individual variation in aggression of feral rodent strains: A standard for the genetics of aggression and violence? Behav Genet 33: 485–501.

79. Miczek KA, Almeida RMM de, Kravitz EA, Rissman EF, Boer SF de, et al. (2007) Neurobiology of escalated aggression and violence. J Neurosci 27: 11803–11806.

80. Fuxjager MJ, Oyegbile TO, Marler CA (2011) Independent and additive contributions of postvictory testosterone and social experience to the development of the winner effect. Endocrinology 152: 3422–3429.

81. Schwartzer JJ, Ricci LA, Melloni RH Jr (2013) Prior fighting experience increases aggression in Syrian hamsters: implications for a role of dopamine in the winner effect. Aggress Behav 39: 290–300.

82. Staffend NA, Meisel RL (2012) Aggressive experience increases dendritic spine density within the nucleus accumbens core in female syrian hamsters. Neuroscience 227: 163–169.

83. Wang Y-F, Hatton GI (2007) Interaction of extracellular signal-regulated protein kinase 1/2 with actin cytoskeleton in supraoptic oxytocin neurons and astrocytes: role in burst firing. J Neurosci 27: 13822–13834.

84. Calcagnoli F, de Boer SF, Beiderbeck DI, Althaus M, Koolhaas JM, Neumann ID (2014) Local oxytocin expression and oxytocin receptor binding in the male rat brain is associated with aggressiveness. Behav Brain Res 261: 315–322.

85. Toth M, Tulogdi A, Biro L, Soros P, Mikics E, et al. (2012) The neural background of hyper-emotional aggression induced by post-weaning social isolation. Behav Brain Res 233: 120–129.

86. Veenema AH, Torner L, Blume A, Beiderbeck DI, Neumann ID (2007) Low inborn anxiety correlates with high intermale aggression: Link to ACTH response and neuronal activation of the hypothalamic paraventricular nucleus. Horm Behav 51: 11–19.

87. Toth M, Fuzesi T, Halasz J, Tulogdi A, Haller J (2010) Neural inputs of the hypothalamic "aggression area" in the rat. Behav Brain Res 215: 7–20.