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Pup Exposure Elicits Hippocampal Cell Proliferation in the Prairie Vole

Michael G. Ruscio[1,*], **Timothy D. Sweeny**[2], **Julie L. Hazelton**[3], **Patrin Suppatkul**[4], **Emily Boothe**[1], and **C. Sue Carter**[4]

1*Department of Psychology, College of Charleston, Charleston, SC 29401*

2*Department of Psychology, Northwestern University, Evanston, IL 60208*

3*Department of Anesthesiology, University of Maryland School of Medicine, Baltimore, MD 21201*

4*Brain Body Center, Department of Psychiatry, University of Illinois at Chicago, Chicago, IL 60614*

Abstract

The onset of parental behavior has profound and enduring effects on behavior and neurobiology across a variety of species. In some cases, mere exposure to a foster neonate (and a subsequent parental response) can have similar effects. In the present experiment we exposed adult male and female prairie voles (*Microtus ochrogaster*) to two foster pups for twenty minutes and quantified cell proliferation in the dentate gyrus of the hippocampus (DG), medial amygdala (MeA) and cortical amygdala (CorA). Prairie voles are highly social rodents that typically display biparental care and spontaneous parental care when exposed to foster pups. Comparisons were made between the animals that responded parentally or non-parentally towards the pups, as well as control conditions. Cell proliferation was assessed using injections of 5-bromo-2'-deoxyuridine (BrdU) and immunocytochemical localization of this marker. The phenotype of the cells was determined using double label immufluoresence for BrdU and TuJ1 (a neuronal marker). An increase in cell proliferation in the DG was seen in animals exposed to pups. However, animals that responded non-parentally had a greater number of BrdU labeled cells in the DG compared to those that responded parentally. The majority of BrdU labeled cells co-expressed TuJ1 across all groups. These results demonstrate that exposure to a foster pup, and the behavioral reaction to it (parental or non-parental) is associated with site-specific changes in cell proliferation.

Keywords

prairie vole; dentate gyrus; neurogenesis; parental behavior; medial amygdala

INTRODUCTION

Pregnancy and parturition cause significant changes in the female brain and behavior [22,31] including changes in neurogenesis [52]. Pregnancy is preceded by a number of social

Correspondence: Michael G. Ruscio, Department of Psychology, College of Charleston, George St., Charleston, SC 29424, Phone: 843-953-7106; FAX: 843-953-7151, e-mail: E-mail: rusciom@cofc.edu.

interactions (such as mating, and in some species pair bonding) and accompanied by significant neuroendocrine changes [37,49]. Although exempt from gestation and birth, males (particularly in bi-parental species) experience neuroendocrine changes coincident with, or preceding the birth of their offspring [23,60]. This cascade of events in females and males typically results in an entirely new set of behaviors to ensure the survival of their offspring [4]. However, in several species, mere exposure to foster neonatal con-specifics can elicit parental behavior not qualitatively different from that seen in natural parents (with the exception of lactation) [29,47]. Furthermore, the pattern of neural activation seen following induction of parental behavior in female rats resembles that seen in post-partum female rats [36]. This phenomenon of sensitization exemplifies how rapidly prominent behavioral changes occur through the exclusive set of stimuli a neonatal pup provides. Additionally, it allows for the study of neural changes associated with neonate exposure and subsequent parental behaviors apart from the neuroendocrine changes of gestation, parturition and lactation.

Distinct populations of prairie voles are naturally bi-parental and alloparental [12,48]. Additionally, a large percentage of adult virgin prairie voles (males and females) exposed to foster pups show spontaneous parental behavior, including retrieval, grooming and arched-back nursing posture [30,47]. Furthermore, prairie voles exposed to pups experience lasting changes in social behavior including a facilitation of subsequent parental responsiveness [48]. Cell proliferation resulting in the production of neurons (neurogenesis) or glial cells (gliogenesis) may be a neural mechanism that responds to pup exposure and underlies behavioral changes. In the present experiment we determine if parental behavior, elicited through exposure to a foster pup, increases neurogenesis in prairie voles (*Mircrotus ochrogaster*).

Adult neurogenesis occurs in several mammalian species [reviewed in 19] including prairie voles [8,10]. Typically, the birth of new cells occurs in proliferative zones; the dentate gyrus of the hippocampus (DG) and the subventricular zone (SVZ) and in many cases these new neurons become functional [15,50,54,58]. Hippocampal neurogenesis may play a role in learning, though the precise nature of the relationship is not yet determined [28]. Similarly, adult cell proliferation and neurogenesis, particularly in the DG may be related to the processing of novel information [2,18,53]. Social interactions with unfamiliar conspecifics can increase neurogenesis [7]. Environmental enrichment (which often includes increased social interactions with conspecifics) also leads to increases in neurogenesis, primarily in the DG [reviewed in 39]. Increased exercise available in these enriched environments is likely to be a crucial component in the increased neurogenic response [57,59]. Conversely, aversive social stimuli such as predator odors [56] subordination stress [13] and resident-intruder [14] stress can decrease hippocampal cell proliferation and neurogenesis. Social isolation results in significant decreases in cell proliferation and neurogenesis as well [6,32,55].

Female prairie voles show increased neurogenesis following exposure to a conspecific male [10]. Changes in gonadal hormones associated with these interactions also may contribute to these alterations in neurogenesis [8,9, reviewed in 11]. In addition to established proliferative zones (DG and SVZ) cell proliferation in the amygdala is responsive to alterations of social environment and hormonal treatments in prairie voles [8,9,10]. Based upon the existence of proliferation in this nucleus and its well established role in social behaviors, including parental behaviors, we performed measurements within select subdivisions (medial and central nuclei) of the amygdala in addition to the DG and SVZ.

In the present experiment we briefly presented foster pups to virgin adult male and female prairie voles. We predicted that pup exposure would be perceived as a generally positive social interaction (among the animals that respond parentally to the pup) and would result in an increase in neurogenesis in our regions of interest, particularly the DG. We also consider

behavioral reaction of our subject to the stimulus. It is possible that the display of parental behaviors, or their absence may be associated with differences in rates of cell proliferation or neurogenesis. We differentiate among new born cells by using markers for cell birth and phenotypic identification of cells (neurons or glial cells).

METHODS

Subjects

Subjects were laboratory-bred male and female prairie voles, approximately 60 days of age and descendants of a wild stock originally caught near Champaign, Illinois. Our stock was systematically outbred. Prairie voles were maintained on a 14:10, light:dark cycle and allowed food (Purina high-fiber rabbit chow) and water *ad libitum*. Animals were housed in same sex pairs (29 cm × 19 cm × 12.7 cm cages) from weaning (at 21 days of age) until testing and sacrifice. Animals in all conditions were housed in a single-sex colony room. All husbandry and experimental procedures were approved by an IACUC committee, ACC no. 04-078.

Behavioral Testing

At approximately 60 days of age male and female voles were behaviorally tested. All animals were given 30 minutes habituation in the testing cage (29 cm × 19 cm × 12.7 cm) prior to any manipulation. Each of the following testing conditions lasted 20 minutes.

Pup Exposure—Two 1–3 day old stimulus foster pups were placed in the testing cage in the end of cage opposite the subject. Subjects in this condition were further classified, as either parental or non-parental, based upon their reaction to the pups.

Pup Exposure, Parental: Animals that approached, retrieved, groomed and displayed crouching posture over both pups were classified as parental. Latency to approach either pup was recorded at the first approach that was coupled with an olfactory investigation and immediate proximity to the pup. Latency to retrieval of either pup was quantified as an adult vole using its mouth to pick up a pup from the distal corner of the cage and bring it to the opposite side of the cage. Latency to crouching posture was marked when both pups had been retrieved and the adult remained relatively stationary, crouching over both pups. Duration of crouching was the total time during the 20 minute trial the adult crouched over both pups. Crouching likely serves to protect the pups, regulate their body temperature and facilitate nursing (when displayed by lactating dams).

Pup Exposure, Non-parental: Animals that approached the pups then attempted to bite them were considered non-parental. The testing was stopped after the first attempted biting to avoid potential harm to the pups. The latency to approach pups and the latency to attempted biting was recorded.

Tootsie Roll—Two tootsie rolls were unwrapped and placed in the testing cage, opposite to the subject. The latency to approach the tootsie roll was recorded. This condition controlled for exposure to a novel object of similar size and a novel odor.

No Pup—The experimenter placed his or her hand in the testing cage at the beginning and end of 20 minute test. This condition controlled for any effects caused by handling and placement in a novel (prior to habituation) test arena.

Assignment to pup exposure, tootsie roll or no pup condition was random.

BrdU injections

BrdU (5-bromo-2'-deoxyuridine) is an established marker of cellular proliferation. BrdU is incorporated in the DNA while cells are in the S-phase of the cell cycle. BrdU was prepared the day of injection by dissolving BrdU (Sigma-Aldrich, #B5002) in a 0.9% saline solution with NaOH (0.007%) to a concentration of 20mg of BrdU/ ml of solution. Subjects received two intraperitoneal injections: one 6 hours following testing, the second 24 hours after testing. Subjects were injected with 0.5ml of solution/100g of body weight (200 mg/kg). This dosage was found to be effective in labeling cells in meadow voles [41]. Figure 1 provides a timeline of our procedure.

Fixation and Sectioning

Animals were euthanized with an overdose of ketamine/ xylazine 48 hours after behavioral testing (24 hours after the second BrdU injection). Brains were removed and fixed using immersion fixation [5]. This procedure includes placing the brains in 20 mls of a 4% paraformaldehyde and 5% acrolein (pH 8.6) solution and gently spinning the tissue for 4 hrs. Brain tissue was then coronally sectioned on a freezing microtome at 40 μm. Sections were collected beginning at the most rostral level of the anterior commissure through the entire hippocampus and most rostral portions of the cerebellum (approximately 100 sections per animal). Sections were placed in cryoprotectant and stored at −20°C until processing for immunocytochemistry. Alternate sections were processed for either BrdU immunocytochemistry (using DAB to visualize cells) or BrdU with TuJ1 double label fluorescent immunocytochemistry to discern cell phenotypes. For all immunocytochemical reactions effort was made to ensure a near equal number of tissue samples from subjects in each condition. Every reaction contained tissue samples from subjects from each condition.

BrdU Immunocytochemistry

Sections were rinsed in KPBS, were denatured in for 1.5 hrs. in a 2M HCl solution at 37 °C, rinsed again in KPBS, placed in a sodium borohydride solution for 20 minutes, rinsed, then blocked with 10% normal rabbit serum. Sections were then incubated in a rat anti-BrdU monoclonal primary antibody (Accurate Chemical, #OBT0030) at a concentration of 1:500 for 1 hr. at room temperature and overnight at 4°C. Sections were then incubated in biotinylated goat anti-rat IgG at 1:200 (Vector) for 1.5 hrs. Staining was further visualized using a standard ABC kit (Vector labs) and 3'- diaminobenzidine (DAB; Sigma). Negative controls of primary only and secondary only, and animals not injected with BrdU were compared with sample tissue from experimental animals (injected with BrdU) prior to collection of any experimental data. The absence of stain was confirmed in control tissue. Liver tissue was also collected from each subject and used as control tissue. BrdU readily labels the chronically proliferating cells in the liver. The presence of BrdU-ir cells in liver tissue was confirmed for each run of immunocytochemistry.

BrdU-TuJ1 Double Label Immunofluorescence

Sections were processed as described above through incubation in the primary BrdU antibody. Following this incubation sections were rinsed in KPBS and then incubated in a rhodamine (RRx) conjugated goat anti-rat antibody (Jackson Immuno Labs) at 1:200 for 1.5 hrs. Sections were then rinsed and incubated in a mouse anti-TuJ1 (Covance, #MMS-435P) primary antibody for 1 hr at room temperature then overnight at 4 °C. TuJ1 is a mouse monoclonal IgG that recognizes a neuron specific class III βtublin, considered to be an early marker for cells that have begun to differentiate into neurons [1,17]. TuJ1 has been used to identify the phenotypes of newly proliferated cells in the adult brain in this species [8,10]. The following day sections were rinsed in KPBS, placed in a FITC conjugated goat anti-mouse antibody (Jackson Immuno Research Labs) at 1:200 for 1.5 hrs. All sections were rinsed and then

mounted using Vector DAPI mounting media. Negative controls and liver tissue were run as they were with immunocytochemistry described above.

Quantification of BrdU Immunocytochemistry

Images were captured using a Nikon Eclipse E 800 microscope, Sensi-cam camera and IP Lab Software®. All photomicrographs for BrdU immunocytochemistry (with DAB) analysis were taken at 100x magnification. Analysis was performed using Image J / NIH Image software. BrdU-ir cell counts of the DG, MeA and CorA were all made from the same section. A representative section was selected from each animal and the sections were matched (across subjects) for rostral-caudal orientation. This section is characterized by the presence of these three regions of interest, as well as the dorsalmedial ascension of the optic tract and the position of the fornix and is approximate to plate no. 58 in Paxinos and Watson [46]. The number of BrdU immunoreactive (BrdUir) labeled cells were counted bi-laterally in the dentate gyrus of the hippocampus (DG), medial amygdala (MeA) and cortical amygdala (CorA). Within the representative section all labeled cells in the DG were counted. The cell counts in the MeA and CorA were made by centering the field of view within either nuclei. All cells within the field of view and within either nuclei were counted. All counts were made by two observers. A subset of sections was stained with cresyl violet to further facilitate the identification of the boundaries of these nuclei. The density of the cells in the subventricular zone (SVZ) was greater and its borders less apparent compared with other regions of interest. For this reason we counted the number of cells within a polygon, 260 × 40 pixels (296.3 microns × 49.3 microns), drawn to include the part of the ventricle where staining was the most dense. The polygon was drawn beginning at the most dorso-lateral edge of the ventricle extending downward. The sample area from the SVZ was approximate to plate no. 32 in Paxinos and Watson [46].

Quantification of BrdU-TuJ1 Double Label Immunofluorescence

Fluorescent images were captured as described above with the addition of using epifluorescent filters for fluorophore excitation. Regions of interest (DG, MeA and CorA) were identified as described above. Additionally, DAPI label on each section facilitated identification of borders of the MeA and CorA. A combination of TRITC (for BrdU label) and FITC (for TuJ1 label) filters were used to capture two separate grayscale images of cellular label. Each image was pseudo-colored (BrdU - red; TuJ1 - green) and merged to identify double labeled cells (which appear yellow in the overlayed image). The total number of BrdU-ir cells was counted in each region as well as double-labeled cells. The percentage of double labeled cells was then calculated. Within the DG an average of 18 BrdU-ir cells per animal were quantified. Within the MeA and CorA an average of 8 BrdU-ir cells per animal were quantified.

Statistical Analyses

All statistical procedures were performed using SPSS 13.0, with the significance level of $p < 0.05$ for all tests. If the significance level was $p < 0.01$ this was noted. All data were tested for assumptions of normality and equal variance. T-tests were used to determine any sex differences in parental or non-parental responses in the pup exposure conditions. MANOVA (with sex and group as independent variables) analyses were used on data from BrdU immunocytochemistry and double label immunofluorescence. Fisher's LSD post hoc tests were performed if the overall MANOVA was significant.

RESULTS

Behavior

Twenty–six voles were placed in the pup exposure condition: 16 voles responded parentally (6 females and 10 males) and 10 voles responded non-parentally (5 females and 5 males).

Among the parental animals the mean latency to approach either pup was 56 ± 16s (± SE). The mean latency to retrieval (picking up a pup and typically returning it to a corner) was 131 ± 28s (± SE). The mean duration spent huddling over both pups in kyphotic posture was 854 ± 63s (± SE). There were no statistically significant sex differences in any of the above parental behaviors. All of the non-parental animals behaved aggressively towards the pups. Within this group the mean latency to an attempted bite of either pup was 145 + 37s (± SE). Females who attempted to bite the pups had a significantly shorter mean latency (64 ± 19s) than males (213 ± 52s), ($t = -2.44$, $df = 9$, $p < 0.05$). Testing was stopped immediately after the first attempted bite to ensure the pups were not harmed.

BrdU Immunocytochemistry

DG—Behavioral testing condition had a significant effect on the number of BrdU-ir cells in the DG ($F_{(3,37)} = 5.40$, $p < 0.05$). The animals in the no pup condition had significantly fewer labeled cells than all other conditions. Animals in the parental condition had significantly fewer labeled cells than the non-parental condition (but not the tootsie roll condition) (Figure 2 and Figure 3). There was no sex difference ($F_{(1,37)} = 0.29$, $p = 0.58$).

MeA—The number of BrdU-ir cells in the MeA was not significantly affected by behavioral testing condition ($F_{(3,30)} = 1.34$, $p = 0.28$) or the sex of the subjects ($F_{(1,30)} = 1.51$, $p = 0.23$) (Figure 4).

CorA—The number of BrdU-ir cells in the CorA was not significantly affected by behavioral testing condition ($F_{(3,31)} = 0.326$, $p = 0.81$) or the sex of the subjects ($F_{(1,31)} = 0.00$, $p = 0.99$) (Figure 4).

SVZ—The number of BrdU-ir cells in the SVZ was not significantly affected by behavioral testing condition ($F_{(3,26)} = 0.26$, $p = 0.85$) or the sex of the subjects($F_{(1,26)} = 0.04$, $p = 0.84$). The means (+SE) of each group are: no pup 52.25 ± 6.51, tootsie roll 61.0 ± 7.92, parental 55.8 ± 7.12, pup attack 61.13 ±11.42.

BrdU-TuJ1 Double Label Immunofluorescence

The percentages of cells expressing a neuronal phenotype (BrdU/TuJ1 double label) in each area of interest are shown in Table 1. Across behavioral testing conditions the majority of cells were double labeled with BrdU-TuJ1. Images of the double label are shown in Figure 5.

DISCUSSION

Our findings show that pup exposure affects cellular proliferation in the prairie vole. This effect is region specific (within the DG) and dependent upon the behavioral reaction (parental or non-parental) to the pup. Phenotypic identification confirms that the majority (approximately 50–69 percent) of BrdU labeled cells also possess a neuronal marker across conditions.

Among other the rodent species the onset of parental behavior is known to be associated with changes in cell morphology [22,43] and neurogenesis [42,52] within the hippocampus. Furthermore, parity is associated with marked changes in hippocampal dependent memory and spatial tasks [21,24,31,45]. In light of these previous findings, the patterns of cellular proliferation and neurogenesis within the DG demonstrated in the present study are particularly interesting. First of all, these changes are occurring independent of the series of events that would typically precede or accompany the onset of parental care. Additionally, both males and females show a similar pattern of neurogenic responses (further suggesting that the response is not directly related to sex specific hormone patterns associated with parental behaviors). Furthermore, although numerous types of environmental stimuli have been shown to alter

patterns of neurogenesis [reviewed in 33,39], this is a case where the behavioral reaction to the same stimulus resulted in different rates of cellular proliferation and neurogenesis. (The variability in parental responsiveness to a foster neonate was not unanticipated and has been shown in this species as well as several others [29,30,35,48].) The same novel social stimuli of pups produced different rates of cellular proliferation in the DG between the parental and non-parental groups suggesting that the reaction to and the perception of the stimulus is important. Ultimately, the cells that are generated in each of these conditions may have different functional significance.

For example, prairie voles that respond parentally to a brief exposure to a foster pup will show shorter latencies to respond to pups in future parental response testing [48] as do rats [51]. These changes in behavior are not necessarily directly ascribed to hippocampal function, but may be associated with learning and memory formation from an initial parental experience (i.e. exposure to foster pup). To date, the effect of neither parturition nor pup exposure in prairie voles has been tested on the battery of hippocampal dependent tasks as it has been in other rodents. However, prior research demonstrates that cells produced by adult hippocampal neurogenesis most likely become integrated into existing hippocampal circuitry [reviewed in 19], possibly associated with the formation of long term memories. In the parental group, new cells could serve consolidate memories of an initial parental experience. Alternatively, the new cells could be incorporated into circuitry which inhibits aversive responses to the pups, rather than directly activating parental responses.

Assuming that prairie voles that responded non-parentally were experiencing a type of stressful or fearful stimulus the increase in cell proliferation is somewhat unanticipated. Although animals in this condition were essentially aggressive towards the pup, a non-parental response is often attributed to a neophobic reaction including an aversion to pup odors [35]. Other studies demonstrate a decrease in hippocampal neurogenesis or cell proliferation following an aversive or stressful stimulus. However, in the present study, pup presentation is a much more acute stressor (all tests were stopped at the first sign of aggression, typically under four minutes), compared with the chronic stress of isolation [6,32,55] or prolonged exposure (one hour) to predator odor [56] seen in other studies. In the present study adrenal hormones may not have been elevated long enough (or at all) to inhibit neurogenesis. In fact, preliminary data suggest that brief pup exposure reduces plasma levels of corticosterone levels in both parentally and non-parentally responding prairie voles (Bales and Carter, unpublished data).

Nonetheless, it is not apparent why a non-parental response would necessitate the production of more cells relative to the parental condition. If pup exposure is perceived as an acute fearful stimulus its emotional content may serve to facilitate memory; as the role of emotion in memory is well established [25]. However, such effects are largely ascribed to the amygdala, rather than the hippocampus. In future studies, it will be crucial to determine the survival rates of neurons in each of these conditions. If these new cells do survive they may be incorporated into hippocampal circuitry at different rates.

Information regarding cell survival will be particularly important when interpreting the pattern shown by the tootsie roll group. If the tootsie roll group had demonstrated a difference with the parental and/or non-parental groups, it would have indicated a clear difference between a proliferative cellular response to a socially relevant rewarding stimulus and a non-social rewarding stimulus. This not being the case, the increase seen across all three groups (relative to the no pup condition) may be indicative of a response to a novel stimulus; a factor previously associated with an increase in hippocampal neurogenesis [27,28,38]. In addition to the novelty of the stimulus, tootsie roles may have served as a highly rewarding food source, potentially engaging the DG (and related reward circuitry) in a similar fashion as parental behavior (as pups themselves and their behavior can be rewarding stimuli [26]). However, exposure to a

pup clearly modifies future behaviors (parental behavior) in ways a tootsie roll does not. In attempting to understand this behavioral modification we must also consider the possibility that the total number of cells (measured by either proliferation or survival) may not be as relevant compared to where and how the cells are incorporated into existing hippocampal circuitry. Consolidation of a parental experience may require neurogenesis and a unique, but not necessarily greater modification of existing circuitry (i.e. addition of more neurons) relative to tootsie roll or non-parental conditions.

The patterns of cellular proliferation and neurogenesis in the MeA and CorA are also of particular interest. This is not the first report of adult neurogenesis in the MeA and CorA in this species [8,10] and our data confirm neuronal phenotypes in these areas. Although the number of BrdU-ir cells was not significantly different across groups in either the MeA or CorA the apparent increase in BrdU-ir cells in the MeA of the parental group is worth noting. The role of the MeA in parental behaviors, (particularly in processing olfactory stimuli), as well as in individual recognition and discrimination is well established [34,35]. Yet, the precise mechanism which generates new cells in the MeA and their potential incorporation into existing functional circuitry remain poorly understood. New cell growth in this area following parental behaviors suggests modification of this circuitry perhaps facilitating future parental responsiveness through recognition of pup odor as a positive social stimulus. Failure to react to pup odors as aversive stimuli is known to facilitate parental responsiveness (anosmic virgin rats will show shorter latencies to parental behavior [7]). The MeA is undoubtedly active in odor perception in the non-parental group, but does not display the relative increase in cell proliferation. Different behavioral reactions to the pup may indicate different emotional reactions modulated through the MeA and quantifiable through different rates of cellular proliferation. It will be interesting in future studies to assess relative changes in cell death, cell survival as well as potential sex differences in the MeA. The increase in the parental group observed in the current study is largely attributed to the number of BrdU-ir cells in parental males, although females trend in the same direction (and there is not a significant sex difference).

Our measures of the SVZ showed no differences among groups. The SVZ along with DG is another well established proliferative zone. However, it would be inaccurate to assume an identical pattern of results in both the DG and SVZ, as environmental manipulations causing site specific alterations in cell proliferation and neurogenesis is not uncommon [e.g. 3,10]. Alternatively, we must consider that our sample from the SVZ was from a discrete coronal section. A more detailed series of measures which included a broader measure of the SVZ, the rostral migratory stream and olfactory bulb may have yielded different results.

In sum our results show site specific changes in cell proliferation in the DG; an area known to be associated with the processing of socially relevant information. The fact that the patterns of proliferation differ according to discrete behavioral response patterns suggests that the perception of the stimulus may be of particular importance in the modification of this circuitry. Future studies which examine cell survival and cell death will help to elucidate how and if this circuitry is being modified in a functionally relevant manner.

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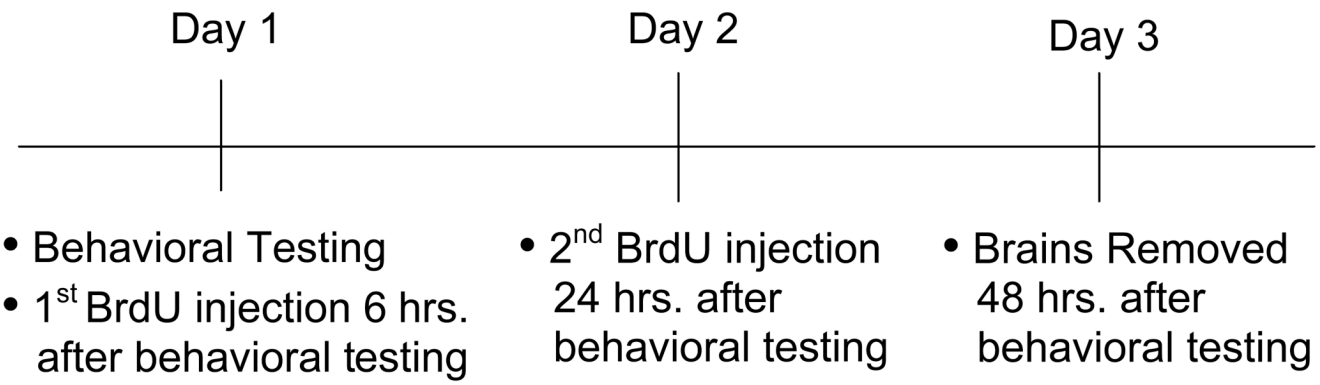

Figure 1.
Schedule of behavioral testing and BrdU injections.

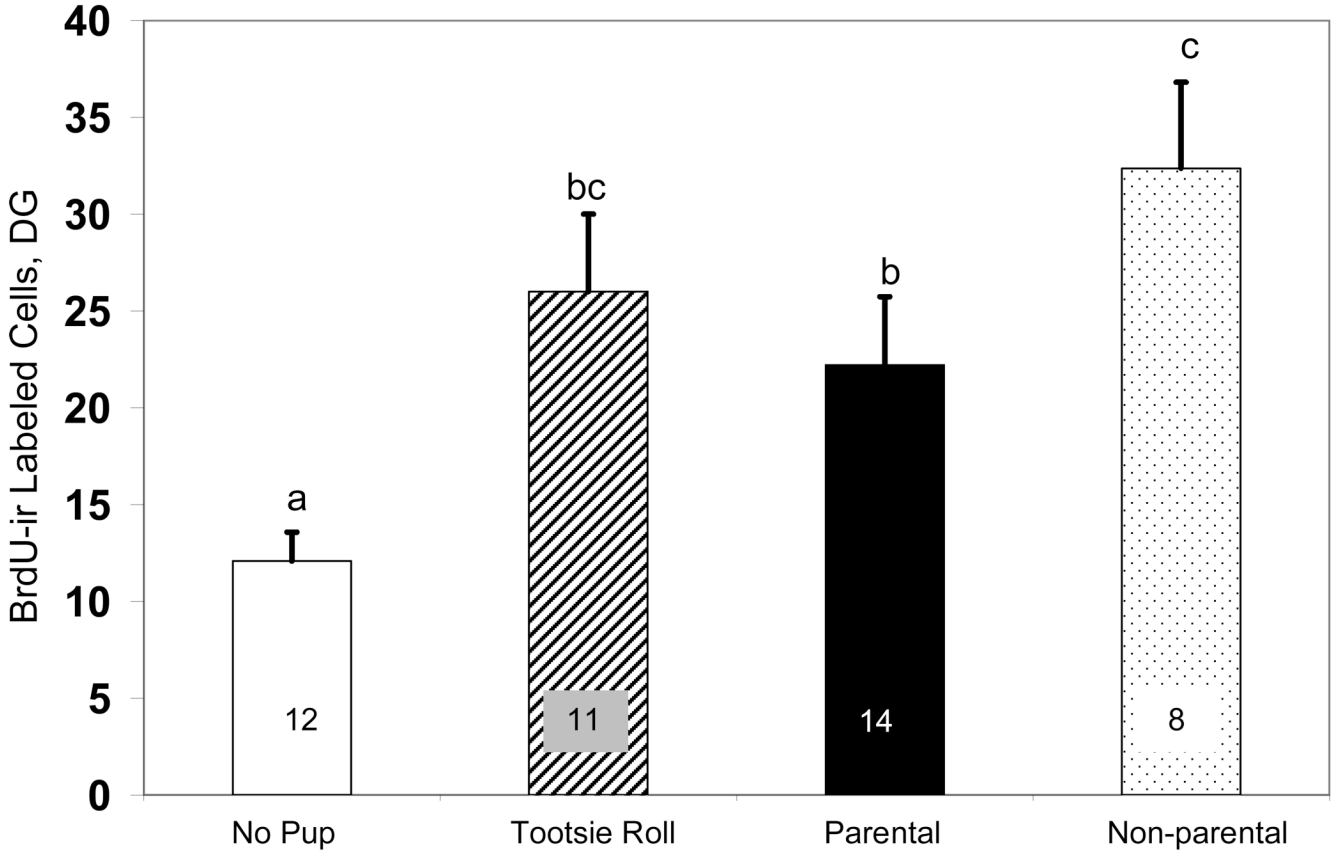

Figure 2.
BrdU-ir in the DG. The parental group had a significantly greater number of BrdU-immunoreactive cells in the DG than the no pup group, significantly fewer than the non-parental group, and showed no difference with the tootsie roll group. Bars represent means + SE. Different letters designate groups that are statistically different at $p < 0.05$. (Tootsie roll group is not significantly different from parental or non-parental groups.) N's are within each bar.

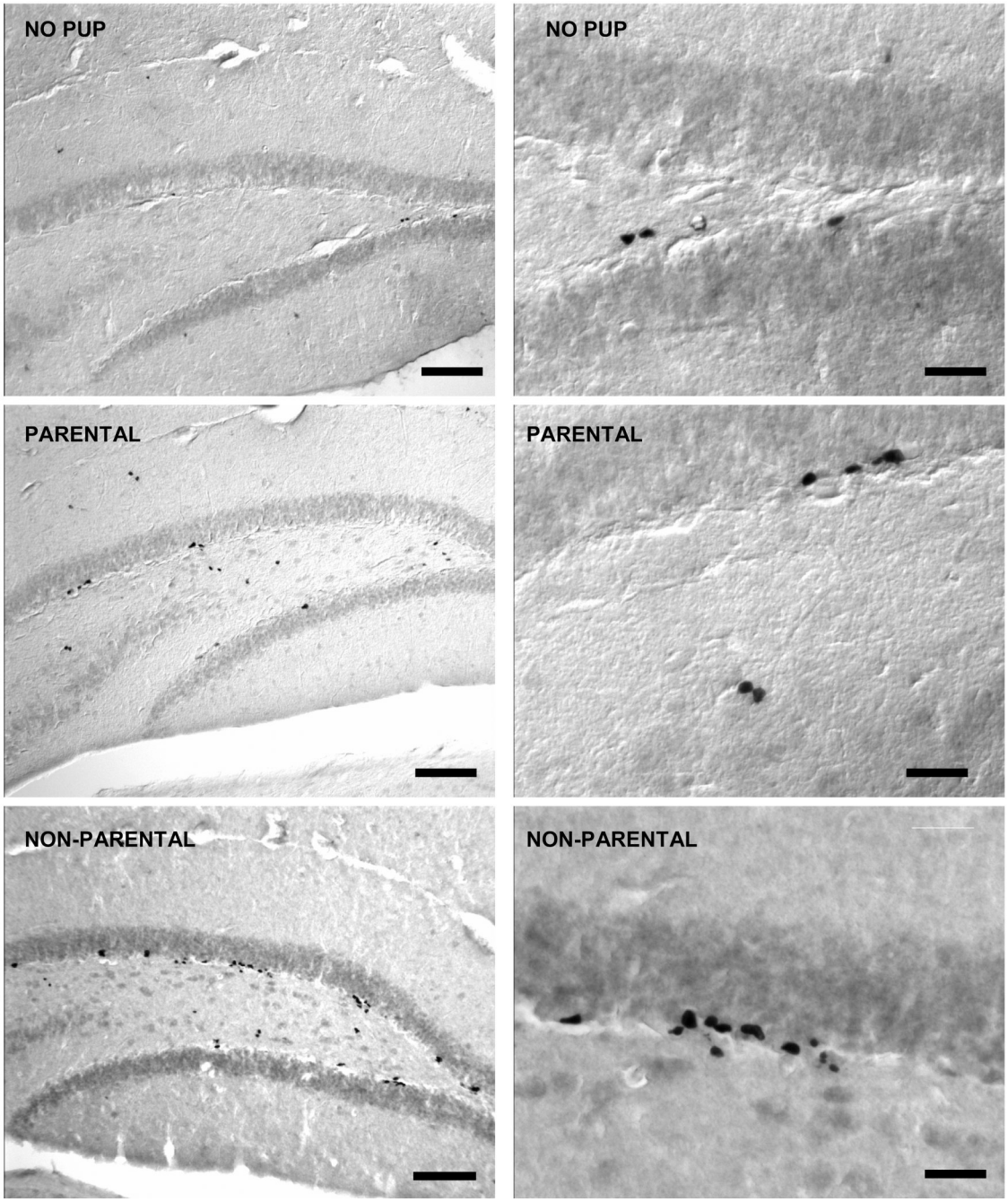

Figure 3.
BrdU-ir in the DG. Panels on the left hand side are taken at low magnification; scale bars = 100 microns. Panels on the right are taken at high magnification; scale bars = 25 microns. Photomicrographs were taken using differential interference contrast.

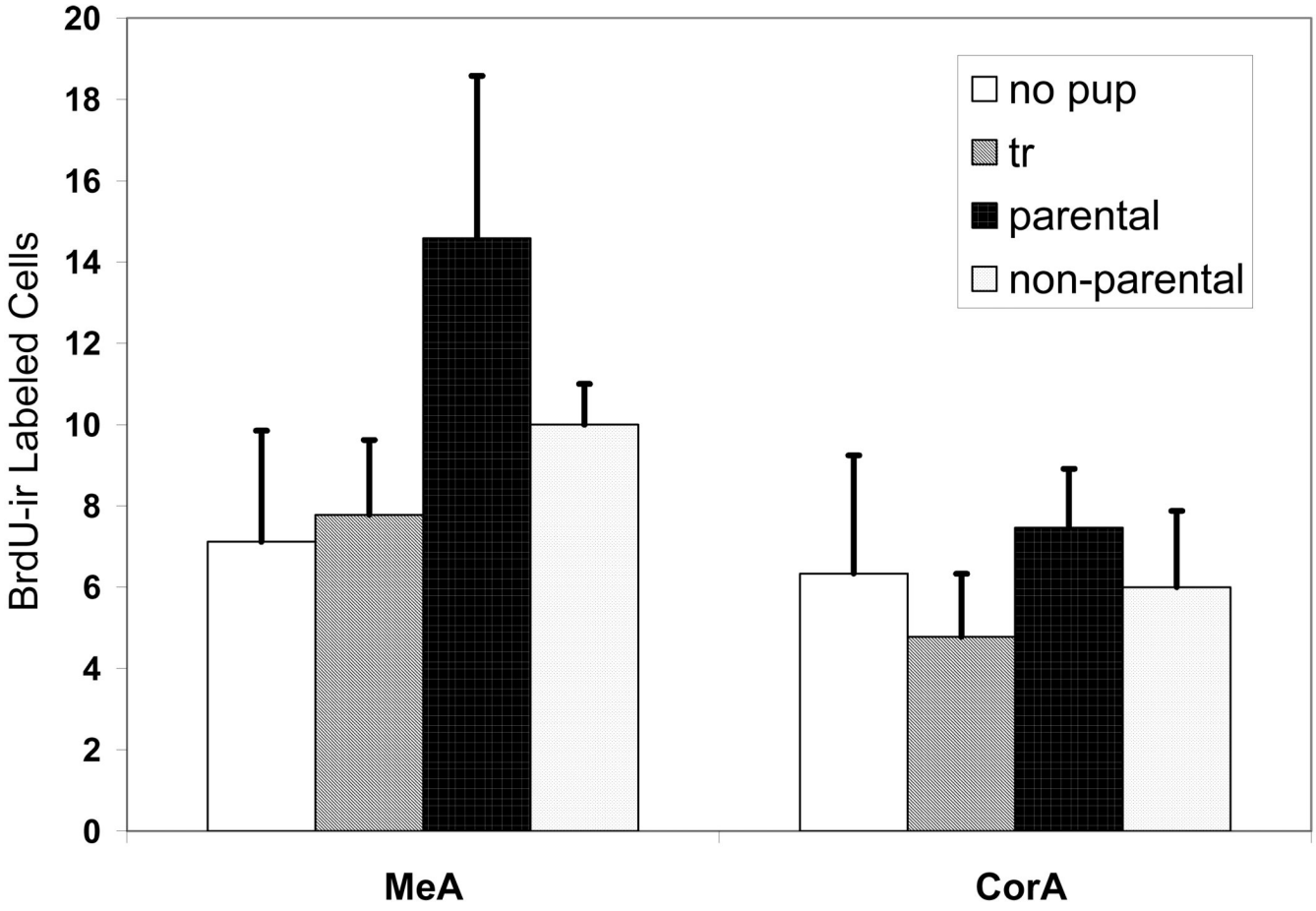

Figure 4.
BrdU-ir in the MeA (left) and CorA (right). Bars represent means + SE. N= 8– 12 per group.

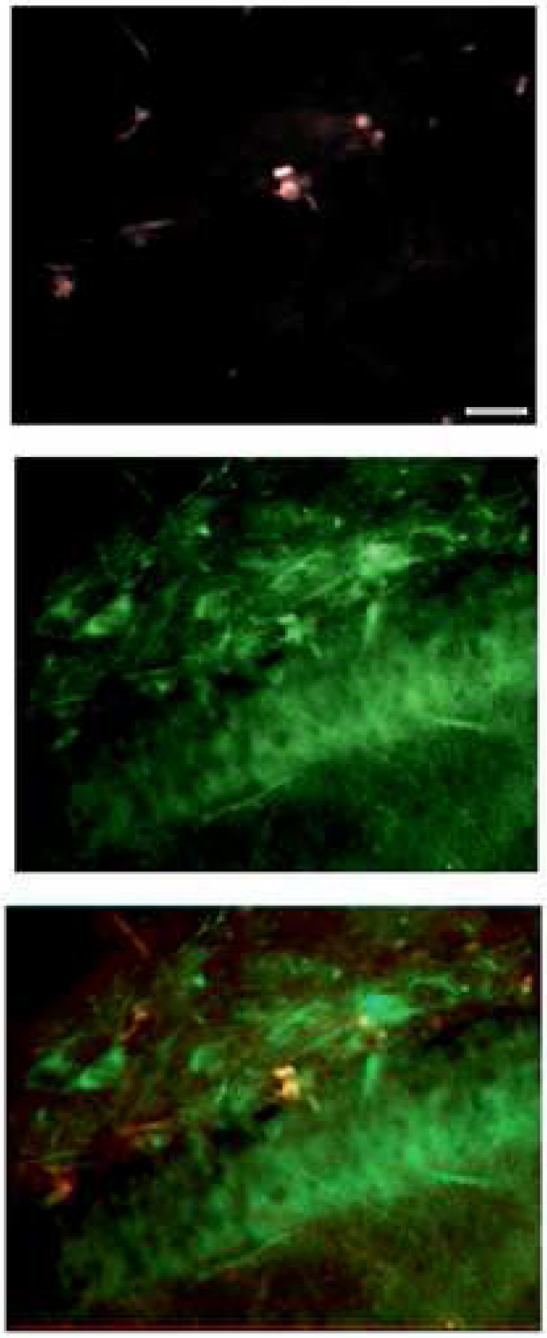

Figure 5.
BrdU-TuJ1 Double Label Immunofluorescence in the DG. BrdU-ir cells, single label (top). TuJ1-ir cells, single label (middle). Merged image showing BrdU/TuJ1 double labeled cells (in yellow) in a parental prairie vole (bottom). Scale bar = 25 microns.

Table 1

Percentage (±SEM) of BrdU-ir Cells Double Labeled with a Neuronal (TuJ1) Marker in the DG, MeA and CorA. N's for each group are in parenthesis.

	No Pup (11)	Tootsie Roll (8)	Parental (10)	Non-parental (7)
DG	66.7 ± 7.2	68.9 ± 5.2	55.1 ± 5.9	50.5± 4.3
MeA	57.7 +12.9	46.4+10.7	55.7+6.4	62.9+15.7
CorA	59.7+11.7	56.7+11.2	46.87+11.9	55.5+15.2