**Effects of Separation Time on Pair Bond Behaviors and Partner-Elicited Neuronal Activation**

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**Abstract**

Humans spend their lifetimes forming selective social bonds and long-lasting relationships, and these bonds are crucial for both mental and physical well-being. Humans will inevitably experience loss of a loved one, and it is necessary to adapt from this loss. An excellent model organism for studying how individuals adapt to partner loss is the prairie vole (*Microtus ochrogaster*). Pair bonding in prairie voles leads to behavioral changes that reinforce the bond which include partner preference and selective aggression. A reversal of these behaviors likely contributes to whether an animal can form a new bond, which we identify as recovery from bond loss. In this thesis, I sought to identify when we can observe recovery from bond loss in male prairie voles. Literature suggests that after 4 weeks of separation from a partner, partner preference and selective aggression are no longer observed, indicating pair bond dissolution and recovery from bond loss. I measured partner preference and selective aggression in male prairie voles after acute (48 hrs) and chronic (4 wks) separation. I hypothesize that adapting to partner loss ultimately leads to a change in how the brain responds to a previous partner. To gain insight into this, I labeled active neurons in the nucleus accumbens (NAc) in response to reunion with a partner, using PS6 (phosphorylated serine-6) as a proxy for neuronal activation. My results show that partner preference and selective aggression are retained in the male prairie voles after acute and chronic separation as neither significantly decreased from a pre-loss state. Additionally, there were no significant changes in partner-elicited neuronal activation in the NAc between acute and chronic separation. These results indicate that my male prairie voles did not lose their pair bond after four weeks of partner separation, which could explain why there were no significant differences observed in partner-elicited neuronal activation in the NAc. These studies provide the foundations for using the prairie vole to model recovery from bond loss.

**Introduction**

Selective social attachments between adults are one of the key behaviors associated with social monogamy, a mating strategy only employed by 3-5% of mammalian species (Carter & Getz, 1993). Social monogamy is characterized by a strong preference for a partner over a stranger, aggression directed only to strangers and not the partner, and biparental care (Carter & Getz, 1993). These behaviors, though animalistic in description, are analogous to human behaviors. Humans spend their lifetimes forming selective attachments and long-lasting relationships (Sadino & Donaldson, 2018). Whether it's a best friend, your favorite family member, or especially a romantic partner, we form strong lasting social bonds with other people. Forming these long-term social bonds is critical for both mental and physical health, buffering against drug misuse, stress, depression, and anxiety ( Lieberwirth & Wang, 2016). When we grieve the loss of a loved one, the body has dampened responses to chronic stress, diminished immune function, and we experience significant detrimental impacts on overall health (Prigerson et al., 1995). In particular, spousal loss is cited as one of life's most stressful experiences, partly due to the fact that for most adults, spouses are our primary attachment (Holmes & Rahe, 1967; Trinke & Bartholomew, 1997). Grieving the loss of a loved one is an arduous and taxing experience but is necessary to recover. For some, this healing process never occurs (Simon, 2013). Long lasting grief is hypothesized to be the result of a failure to incorporate the finality of the loss, or failure to adapt to the loss of a loved one (Field et al., 2005; Shahane et al., 2018).

To better understand how we grieve, we must further explore the neuronal basis of selective attachments. The traditional rodents used in laboratory research, such as rats and mice, are not monogamous and therefore cannot be used to study the selective social attachments that hallmark human romantic relationships. The prairie vole (*Microtus ochrogaster),* however, is a

lab-amenable, socially monogamous rodent and serves as an ideal model organism for studying pair bonds and partner loss. Prairie voles demonstrate the characteristic behaviors of social monogamy such as spending the majority of their time with their partner, defending the nest, and engaging in bi-parental care (Carter & Getz, 1993). We use a partner preference test (PPT) to measure the preference for the partner over an opposite-sex stranger as a proxy for the presence or absence of pair bonds (Carter & Getz, 1993). Studies on prairie voles in the wild show that following the loss of a partner (usually to predation), approximately 80% of partners would not form another pair pond (Getz & McGuire, 1993). What makes these bonds so strong, and how does the prairie vole brain respond to the loss of a partner? Inhibited oxytocin signaling following partner separation is observed in prairie voles, which is hypothesized to drive long-term monogamy by establishing an aversive emotional state in the vole while it's away from its partner. (Bosch et al., 2016). This suggests that spending time with a partner versus a stranger is rewarding, and reward is one of the motivators of social monogamy. Additionally, prairie voles show increased anxiety-like behaviors following separation from their partner (Sun et al., 2014). However, decreased selective aggression and lack of partner preference are observed in male prairie voles after long-term separation from their partner (Sun et al., 2014). Together these studies suggest there are neuromolecular changes that drive hallmark behaviors of monogamy while a pair bond is intact, and neuromolecular changes that occur in response to partner loss. Prairie voles are therefore an ideal model to delineate the biological and behavioral responses to partner loss and how they change over time.

This project seeks to determine the time course of bond loss recovery and to determine what changes in the brain reflect that recovery. The Wang lab has demonstrated that after four weeks without their partner, male prairie voles stop showing partner preference, indicated by

equivalent time spent with a novel, opposite-sex conspecific versus the previous partner (Sun et al., 2014). The Donaldson lab has shown that after four weeks of separation from their partner, male prairie voles are able to form partner bonds with new females that supplant their original pair bond. These studies indicate that male prairie voles recover from bond loss at about four weeks after losing their partner, indicated by loss of partner preference and decreased selective aggression, component behaviors of pair bonds. However, these experiments differ in the time pairs cohabitated before their partner preference was tested, and literature suggests that pair bonds strengthen over time (Scribner et al., 2019). The Wang lab's animals developed their pair bond over 24 hours, whereas the Donaldson lab's animals developed theirs over two weeks. While we know that male prairie voles that formed a pair bond over 2 weeks can form a new bond that supplants the first after four weeks of separation, we don't know if that original bond is actually lost, and that is what this project sought to determine. Additionally, we asked how partner-elicited neuronal activation changes in the nucleus accumbens (NAc), a brain region central to pair bond formation and maintenance, before and after adapting to partner loss. I chose to examine the NAc due to its known role in the reward pathways of the brain. Studies have demonstrated that inhibition of oxytocin and dopamine signaling in the NAc was sufficient to inhibit pair bond formation (Liu & Wang, 2003; L. J. Young et al., 2001). Furthermore, the Donaldson lab has shown robust neuronal activity within the NAc during mating compared to an un-mated control, and mating is a key facilitator of pair bond formation. Therefore, the NAc is likely central to forming and maintaining a partner preference. I used PS6 (phosphorylated serine-6) as a proxy for neuronal activation. PS6 is a phosphorylation marker found only on active ribosomes, therefore it can be used as a marker for neuronal activity. Further, it co-expresses with *c-fos,* a well-validated  marker for neuronal activation (Knight et al., 2012). Past

studies have shown that activity markers can delineate specific neuronal activity involved in different behaviors and responses to specific stimuli (Curtis & Wang, 2003; Knight et al., 2012). Using immunohistochemistry to label PS6 positive neurons, we can identify differential activity in the NAc of prairie voles before and after pair bond dissolution.

I anticipated that males allowed to cohabitate with a partner for two weeks would lose partner preference following chronic (4 weeks) separation, and that there would be less partner-elicited neuronal activation in the NAc following chronic partner separation. Partner preference and partner-elicited active neurons will be compared between acute (48 hours) and chronic (4 weeks) separation. Pair bonds should still be intact at the acute timepoint, and therefore provides a comparison of partner preference and active neurons impacted by duration of separation, rather than just the stress of separation.

**Experimental Design**

*Experiment One: Confirming Bond Loss after Chronic Separation*



**Figure 1.** Experimental timeline for the behavioral study. (Courtesy Julie Sadino)
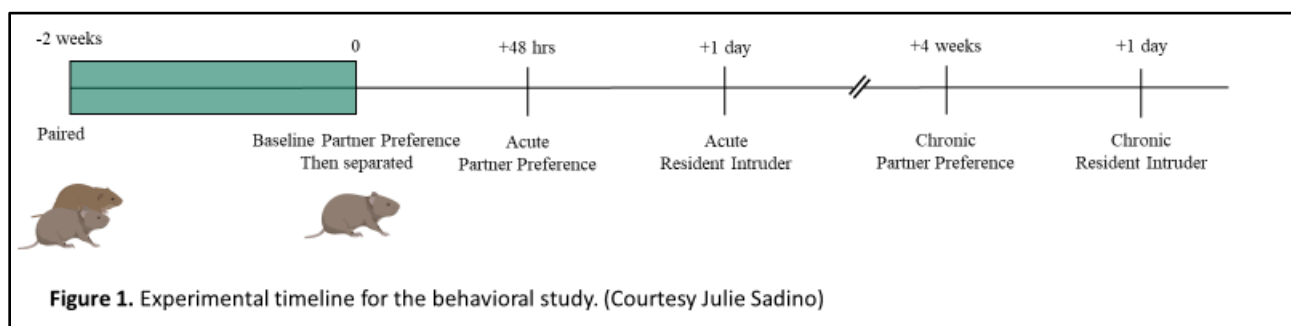
In this experiment (n = 16), I allowed 16 pairs of voles to cohabitate for two weeks. At the end of the two weeks, I measured partner preference using a PPT to obtain baseline partner
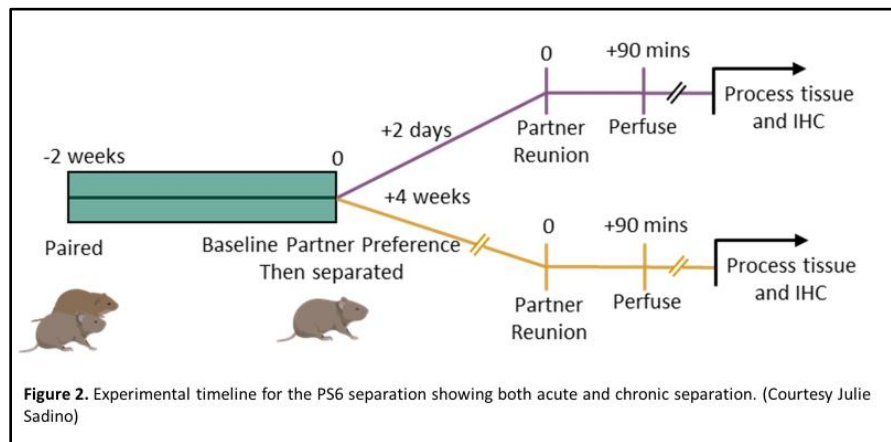
preference. Voles were then separated for 48 hours (acute). Following 48 hours of separation,

acute partner preference was measured again using a PPT. 24 hours following that, males were

given an acute *resident intruder test* to measure same-sex-directed aggression, another

component behavior of pair bonded voles. Immediately following the acute PPT, the voles

remained separated for an additional 4 weeks for chronic separation to dissolve the pair bond.

Chronic partner preference was measured using a PPT, and 24 hours later, the males were given

a chronic *resident intruder test.*

*Experiment Two: PS6+ Neurons in Acute or Chronic Separation*

In this experiment, voles (n = 8) were paired and cohabitated for two weeks. At the two-

week mark, they were given a partner preference test to ensure a pair-bond was formed.

Immediately following the partner preference test, the pairs were separated into clean cages and

singly housed for either 48 hours or 4 weeks. This difference in separation will show how the brain adapts following partner separation while the pair-



Figure 2. Experimental timeline for the PS6 separation showing both acute and chronic separation. (Courtesy Julie Sadino)

bond is still intact to when it is hypothesized to decay. Following the separation, these voles were

reunited with their original partner for 90 minutes, and then the males' brains were perfused.

Brain tissue was sliced, stained for PS6 using immunohistochemistry, and PS6+ neurons were

hand-counted.

**Methods**

*Subjects*

The prairie vole colony was established by breeding together voles from a colony at the University of California Davis and from a colony Emory University. The breeding of these two colonies was meant to increase genetic diversity between subjects. Voles were weaned from their parents at twenty-one days post-natal and cohabitated in groups of four with same sex siblings and/ or same sex pups weaned within a similar time frame. The colony was kept on a 14:10 hour light/ dark cycle in a humidity-controlled room. All voles used were allowed to reach sixty days of age before being involved in any experiment or procedures. All procedures were approved under the University of Colorado's *Institute of Animal Care and Use Committee* (IACUC) protocol 2435 and followed standard quality of care guidelines set by the *National Institutes of Health* (NIH).

*Tubal Ligations*

All females (n = 42) were tubally ligated to avoid any confounds of pregnancy, but to keep the ovaries intact as to not impact hormonal function. Females were weighed and given 4.0 mg/ kg of Meloxicam SR (Zoopharm, Laramie, WY) by subcutaneous injection to help in pain relief post-surgery. They were anaesthetized using 2% Isoflurane given nasally with $O_2$ gas (Henry Schein, Melville, NY). Depth of anesthetize was monitored by toe pinch. To remove ovaries, a small incision is made on the midline of the lower back. A small cut is made through the body cavity on the left and right sides. The uterus and ovary were pulled through the hole and the fallopian tubes were cauterized prior to suturing the incisions. The skin incision of the back was

closed with surgical staples and an even mixture of lidocaine (Dynarex, Orangeburg, NY) and triple-action antibiotic (Dynarex, Orangeburg, NY) was placed onto the sealed wound. Locomotion, breathing, and sudden changes in appearance were monitored during and for 30 minutes post-surgery. Females were monitored once a day, for three days for pain and wound closure. Staples were removed 10 days post-surgery.

*Pairing & Cohabitation*

One female was paired with one male, both of which were between the ages of 60 and 180 days. Pairs were determined by proximity in age and lack of same parents. Voles were placed from their homecage into a smaller cage with their partner with fresh bedding, food, water bottles, a cotton bedding pad, an igloo and enrichment *ad libidum*. Partners cohabitated undisturbed (except for weekly cage changing) for 2 weeks. Reunion studies did not use fresh cages, and instead the female was placed into her partner's cage for 90 minutes.

*Partner Preference Tests*

I determined whether the males had formed a pair-bond by a partner preference test at the end of the two-week cohabitation period. The Partner-Preference Apparatus (72 cm *l* x 20 cm *w* x 30 cm *h*) consisted of three chambers that can be separated with wall inserts (K. A. Young et al., 2011). The PPT is filmed for 3 hours with the test male moving between the chambers where his partner and a novel female are tethered to the wall of opposite chambers while his behavior is tracked. Recorded behaviors include distance traveled, time spent alone, with partner, and with the stranger. Both the partner and the novel female are sedated with 2% isoflurane (Henry Schein, Melville, NY) and $O_2$ and given the zip-tie collar. They are monitored for stress,

discomfort, and pain until they show no signs of any of them. The PPT behaviors described above are scored by the *CleverSys* program (v3) and analyzed by an in-house Matlab script (v2).

*Resident Intruder Test*

Resident intruders were naïve males between the ages of 60 and 150 days. Experimental males are left in their homecage and a naïve novel male is placed in the cage with them for 10 minutes while being filmed. Following these 10 minutes, resident intruders are placed back into their homecage and allowed to resettle for an hour before they are used in the next test. Huddling, jumping, investigation, tumble fighting, rearing, and auto grooming were all hand-scored using StopWatch+.

*Cardiac Perfusion*

Following the 90-minute reunion, the males were removed from the partner and immediately anaesthetized with a 1:2 ketamine: xylazine mixture (Akorn, Lake Forest, IL). Once confirmed to be non-responsive using toe and tail pinch, their brains were cleared of blood, fixed, and collected according to standard cardiac perfusion protocol using ice-cold 1X PBS followed by an equal volume of 4% paraformaldehyde (PFA) in 1X phosphate-buffered saline (PBS) (manufacturer). Brains were then left in 4% PFA overnight at 4C prior to being transferred to a 30% sucrose solution for approximately three days or until saturated.

*Tissue Preparation*

Brains are sliced at 50 micrometers on a frozen microtome. These slices are evenly divided in to four groups to ensure consistent staining. One of these groups is separated into a 12-well plate

with maximum 8 slices per well. These slices are rinsed three times in 1X PBS (phosphate-buffered saline, prepared in house), and then incubate in 0.3% Triton-X/1X PBS (Fischer BioReagents, Hampton, NH) with 10% Normal Donkey Serum (NDS) (Jackson ImmunoResearch, West Grove, PA) for 2 hours. Then these slices incubate in a 1:500 dilution of the primary antibody, Rabbit anti-PS6 (Invitrogen, Carlsbad, CA) suspended in a 0.3% TWEEN/ 5% NDS solution, for 48 hours. The slices are then rinsed three times in 1X PBS, and then incubate for two hours in a 1:500 dilution of secondary antibody, Biotinylated Donkey anti-rabbit in 0.3% TWEEN (Fischer BioReagents, Hampton, NH). Following this incubation, the slices are then rinsed three times in 1X PBS, and then incubate in a 1:1000 dilution of Streptavidin (to bind to the biotin) conjugated to horseradish peroxidase (HRP) (Abcam, Cambridge, UK) for one hour. Then the slices are once again rinsed, treated with the DAB (3,3' Diaminobenzidine) systems (Fischer Scientific, Hampton, NH) for one minute, rinsed in DI water twice, and then mounted on microscope slides with Moweol (prepared in house). Finally, the slices incubate in a DAPI (Life Technologies, Eugene OR) stain to visualize all neurons within the slices.

Following staining, the slices are mounted on Superfrost Plus glass slides (Thermofischer, Waltham, MA), cover-slipped (Globe Scientific, Mahwah, NJ), and sealed with clear nail polish (Electron Microscopy Sciences, Hatfield, PA) 24 hours later. Slides are maintained at 20$^{\circ}$C.

*Data Analysis*

Partner preference data and open-field test data is analyzed using *CleverSys (*v3), a program designed to automate and standardize behavioral analysis. Locomotion, time spent with either partner or stranger, and time spent alone are all outputs of CleverSys (v3). This data is then used

to determine if there is partner preference using an in-house Matlab code (v2). Locomotor data and time spent in center/outside is used as a metric for anxiety in the case of the open-field test.

Tissue analysis is done by hand counting stained neurons in each tissue slice, and reporting active neurons compared to total neurons in the NAc. These measurements are used as the primary comparison for between-group and within-group comparisons.
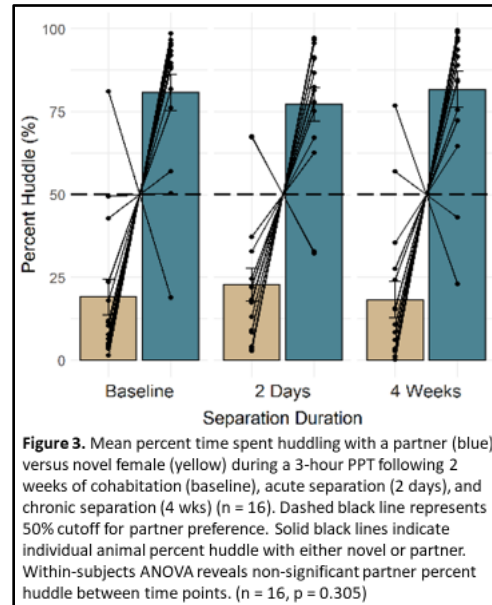
*Statistical Analysis*

Partner preference was analyzed using a t-test against a mean of 50% for partner preference huddle. Time spent with partner versus a stranger at each time point was analyzed using a two-way ANOVA, and percent huddle time with a partner at each time point was analyzed using a Repeated Measures one-way ANOVA. Resident Intruder Test behaviors were analyzed across acute and chronic separation using paired t-tests. PS6+ neurons across the two time points was analyzed by an independent-samples t-test.
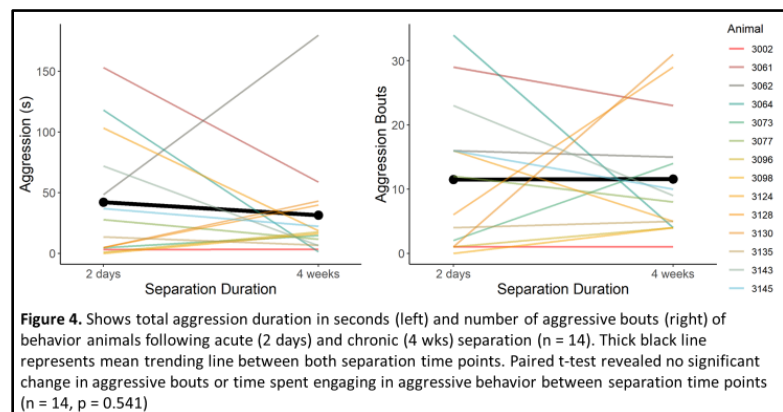
**Results**

**Experiment One: Four weeks was not sufficient for pair-bond abolishment in our prairie voles**

I anticipated our voles to have partner preference following acute separation, and no partner preference following chronic separation. Contrary to my hypothesis, after chronic separation, there was no significant (p = 0.305) decrease in partner preference from baseline or acute separation (Figure 3). A reapeated-measures ANOVA revealed a non-significant difference in partner preference between separation time points (n = 16, p = 0.305) (Figure 3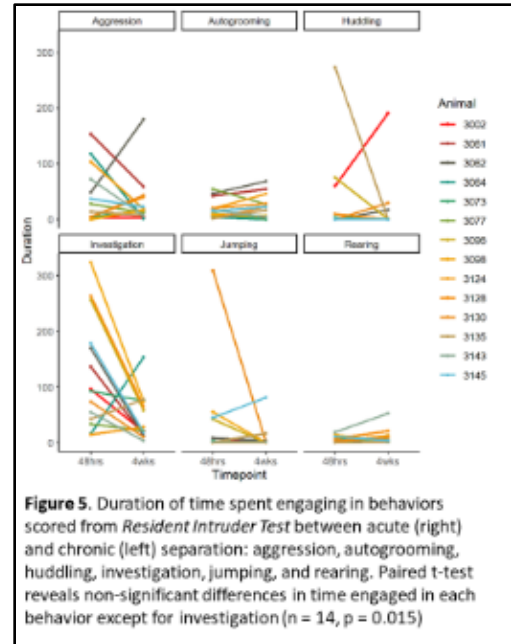). Partner preference is observed at all three time point as determined by a t-test against a mean of 50%. Partner preference in this study was determined by percent time spent huddled with a partner divided by total time spent huddled with a female, with preference being more than 50% time spent with the partner.



**Figure 3.** Mean percent time spent huddling with a partner (blue) versus novel female (yellow) during a 3-hour PPT following 2 weeks of cohabitation (baseline), acute separation (2 days), and chronic separation (4 wks) (n = 16). Dashed black line represents 50% cutoff for partner preference. Solid black lines indicate individual animal percent huddle with either novel or partner. Within-subjects ANOVA reveals non-significant partner percent huddle between time points. (n = 16, p = 0.305)

Resident Intruder data was hand scored over a 10-minute test. I anticipated there to be a significant decrease in aggressive behaviors following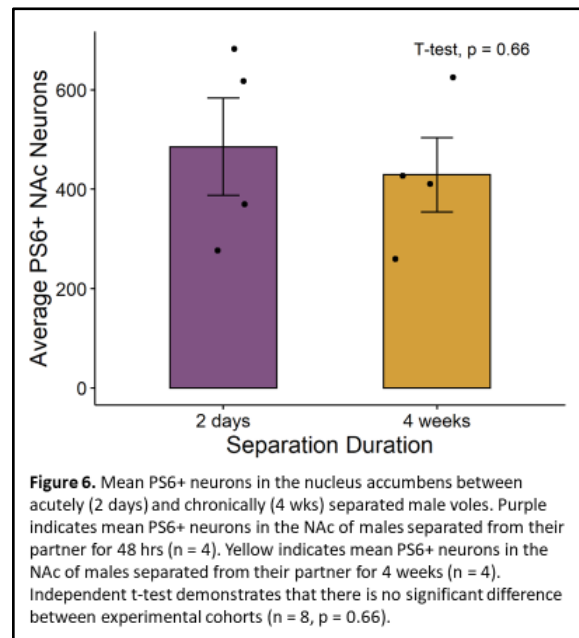 chronic separation. Two animals were excluded in the analysis as outliers. A paired t-test reveals a non-significant decrease in male aggression between acute separation and chronic separation (n = 14, p = 0.541) (Figure 4). A



**Figure 4.** Shows total aggression duration in seconds (left) and number of aggressive bouts (right) of behavior animals following acute (2 days) and chronic (4 wks) separation (n = 14). Thick black line represents mean trending line between both separation time points. Paired t-test revealed no significant change in aggressive bouts or time spent engaging in aggressive behavior between separation time points (n = 14, p = 0.541)

paired t-test also reveals non-significant changes in other scored behaviors: autogrooming, huddling, jumping, and rearing. However, investigation showed a significant decrease between acute and chronic separation (n = 14, p = 0.015) (Figure 5). While this data does not match my hypothesis, it reflects what we see in the partner preference data, supplementing the conclusion that the pair bonds did not dissipate following chronic separation.
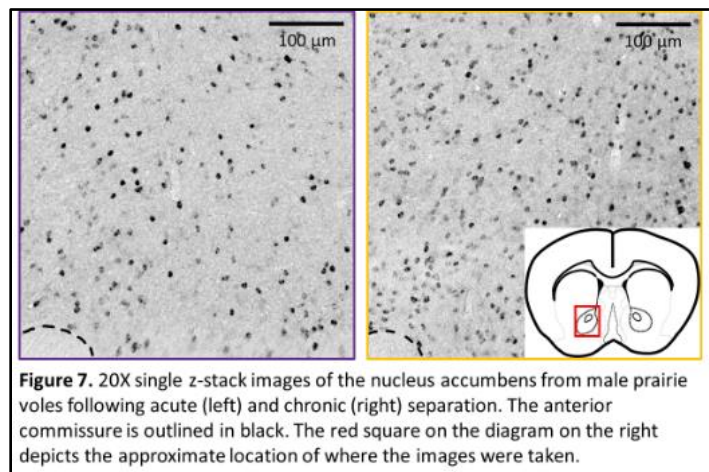


**Figure 5**. Duration of time spent engaging in behaviors scored from *Resident Intruder Test* between acute (right) and chronic (left) separation: aggression, autogrooming, huddling, investigation, jumping, and rearing. Paired t-test reveals non-significant differences in time engaged in each behavior except for investigation (n = 14, p = 0.015)

**Experiment Two: There are no significant differences in PS6+ neurons in the NAc between acutely and chronically separated prairie voles.**

I hypothesized there to be significantly fewer PS6+ neurons in the NAc following reunion of chronically separated pairs versus acutely separated pairs. An independent t-test reveals a non-significant difference (n = 8, p = 0.66) in the number of active NAc neurons between separation time points (Figure 6). These neurons were hand counted in a single plane image of the NAc using a grid system to capture the same area between brains (Figure 7).



Figure 6. Mean PS6+ neurons in the nucleus accumbens between acutely (2 days) and chronically (4 wks) separated male voles. Purple indicates mean PS6+ neurons in the NAc of males separated from their partner for 48 hrs (n = 4). Yellow indicates mean PS6+ neurons in the NAc of males separated from their partner for 4 weeks (n = 4). Independent t-test demonstrates that there is no significant difference between experimental cohorts (n = 8, p = 0.66).

**Discussion**

This study shows that under our experimental conditions, our prairie voles do not lose their pair bonds after 4 weeks of separation as measured by



Figure 7. 20X single z-stack images of the nucleus accumbens from male prairie voles following acute (left) and chronic (right) separation. The anterior commissure is outlined in black. The red square on the diagram on the right depicts the approximate location of where the images were taken.

partner preference and selective aggression. Though other behaviors scored during the resident intruder tests showed no significant change between acute and chronic separation, investigative behavior decreases significantly from acute to chronic separation. Additionally, I demonstrate that there is no significant difference in the number of partner-elicited active neurons in the NAc of male prairie voles who have been acutely and chronically separated from their partner.

My results could differ from the work shown by the Wang and Donaldson labs for a few key reasons. First, my experiment was a within-animals study, meaning my pairs were reintroduced at 48 hours and at 4-weeks, while the Wang lab only allowed reintroduction at the end of the 4-week separation. This reintroduction may have been enough for the voles to strengthen their pair-bond to outlast chronic separation. This could explain why we see no significant change in partner preference between baseline, acute, and chronic measurements, and no significant differences in aggression between acute and chronic separation. Secondly, my animals cohabitated for two weeks prior to baseline PPT, while the Wang lab's animals only cohabitated for 24 hours (Sun et al., 2014). Even though the Wang lab was able to show that their animals had partner preference after 24 hours, the literature suggests that pair bonds strengthen over time (Scribner et al., 2019). While both the Wang and Donaldson lab point to 4 weeks as the pivotal time course to bond loss, the Donaldson lab studies did not solely explore separation; pair bond dissolution could have been supplemented by new partner introduction and cohabitation.

The results from the partner-elicited neuronal activation experiment are contrary to my original hypothesis as well. I hypothesized there to be fewer active neurons in the NAc of chronically separated males compared to acutely separated males because spending time with a partner is less rewarding if the pair bond has dissolved. However, considering my behavioral experiments, it is likely that my animals still had an intact pair bond after 4 weeks of separation. If that were the case, I would not expect to see significant differences in PS6+ neurons between separation conditions. With this in mind, I propose that longer cohabitation led to a stronger pair bond that outlasted chronic separation. I suspect that an original pair bond can be dissolved quicker by introducing a new partner and allowing the new pair to form a pair bond, effectively

replacing the original pair bond. This could explain why the males from the Donaldson lab studies formed secondary pair bonds that supplanted the first following chronic separation.

Additionally, there are limitations of my studies that should be considered in interpreting my data and merit follow up studies. One limitation is that, for the PS6 experiment, we did not perform partner preference tests at the end of each separation condition. Therefore, we do not know if these animals ever lost their partner preference during separation. Additionally, we were not able to process the control group of novel opposite-sex conspecific introduction to determine baseline NAc activity. Therefore, we do not have a measurement of baseline NAc activity due to social interaction and how partner separation alters this baseline activity. For the partner preference experiment, we don't know if partner preference is simply driven by familiarity. Studies must be done with same-sex pair separation and reintroduction to determine if partner preference is driven by remembering a cage mate or is the result of a pair bond. Finally, it is possible that the short reintroduction during the acute partner preference is driving my results and not the duration of initial pairing. Using a within animal study therefore could have inadvertently created a confounding variable that will be explored in future experiments.

Together my experiments suggest that, under our experimental conditions, pair bonds remain intact and neural response in the NAc to partner reintroduction does not change following chronic separation. These results may be impacted by reintroduction between separation conditions or time allotted for pair bond formation. This suggests that while there is something crucial about 4 weeks of partner separation, other factors modulate adaptation to partner loss. Further, these experiments set the stage for future studies that could explore how pair bonds strength is impacted by the duration of cohabitation, or how bond loss recovery can potentially be mediated by introducing a novel animal. Though my results were unexpected, they offer new

and exciting modulators of bond loss recovery to explore. Grief is a complicated and a very human experience. In modeling partner loss in prairie voles, we can begin to explore how the brain adapts while recovering from loss and what factors can disrupt or facilitate this recovery.

**Future Directions**

In order to better understand why my results were so different from my hypothesis, I would like to examine how reintroduction and time spent cohabitating may impact partner preference. To do so, I want to have a cohort of voles cohabitate for two weeks and then be separated for four weeks. I will measure partner preference following the chronic separation. If the pairs do not have a partner preference this could indicate that, in my original experiment, the reintroduction at 48 hours confounded the chronic PPT. I would also like to repeat my behavior study with 24 hours of cohabitation versus two weeks to see how cohabitation duration may have contributed to sustained partner preference following chronic separation. Additionally, I will perform this experiment in females as there are known differences in pair bonding behaviors.

I want to count PS6+ neurons in the nucleus accumbens (NAc) of a naïve baseline control group. This group would consist of naïve males and females introduced for the first time and allowed to interact for 90 minutes before perfusion. This baseline state would provide control data for novelty of interaction after being singly housed. I would also like to count neurons in other areas of the brain important in pair bond formation and maintenance, such as the ventral pallidum, a major output of the NAc, the ventral tegmental area, a major input to the NAc, and both the medial prefrontal cortex and ventral CA1 portion of the hippocampus due to their modulatory signaling with the NAc.

Additionally, this study sits in a larger project that seeks to identify neuromolecular signatures of partner loss. We can immunoprecipitate PS6+ neurons to capture actively translating ribosomes to identify the genetic signature of the active neurons (Knight et al., 2012). This activity dependent pulldown allows us to see which genes become differentially expressed in acute versus chronic separation, as well as in a naïve state. If separation does not significantly impact the number of neurons that become active, perhaps it's the identity of neurons that change in response to separation. The data from immunoprecipitation would offer insight into what molecular mechanisms drive adaptation to partner loss. My mentor, Julie Sadino, has preliminary data identifying a gene expression profile unique to partner loss. Some of the genes she identified are involved in regulating neuronal plasticity and G-protein signaling; which is interesting because literature has identified G-protein coupled receptor ligands essential for pair bond formation and maintenance: dopamine, oxytocin, and vasopressin (Liu & Wang, 2003; Sadino & Donaldson, 2018; Winslow et al., 1993; L. J. Young et al., 2001). Early analysis suggests that the brain adapts following partner loss to accommodate new pair bonds, but there is much to be further explored.

Grief is arduous, poorly understood, yet inevitable. The hope of this study and future studies is to better understand how the brain adapts to the loss of a loved one. Prairie voles offer a model to begin to understand how we can better recover from losing loved ones, and how we can better understand the complexities of selective social attachment.

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