A neuronal signature for monogamous reunion

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Abstract

Pair bond formation depends vitally on neuromodulatory signaling within the nucleus accumbens, but the neuronal dynamics underlying this behavior remain unclear. Using in vivo Ca^{2+} imaging in monogamous prairie voles, we found that pair bonding does not elicit differences in overall nucleus accumbens Ca^{2+} activity. Instead, we identified distinct neuronal ensembles in this region recruited during approach to either a partner or novel vole. The partner approach neuronal ensemble increased in size following bond formation, representing a substrate critically linked to bond formation and maturation.

Keywords: Pair bond, nucleus accumbens, prairie vole, Ca^{2+} imaging

Fewer than 10% of mammalian species, humans included, form mating-based pair bonds[1,2]. Pair bonds are maintained and reinforced over time by a selective desire to seek out and interact with a bonded partner. This behavior is not exhibited by most laboratory rodents, including mice and rats. However, in monogamous prairie voles, pair bonding is easily assessed using a test in which the focal animal chooses between spending time with a pair-bonded partner or a novel opposite-sex vole tethered on opposite sides of an apparatus (Fig 1x)[3,4]. Referred to as a partner preference test, this assay provides an opportunity to examine neural activity while an animal is displaying a pair bond and to ask how behavior and neural activity change as a bond matures.

The nucleus accumbens (NAc) plays a critical role in reward and motivation[5], making it a likely brain region for encoding highly-rewarding pair bonds[6]. When participants in an fMRI study thought that they were holding hands with their pair-bonded partner, rather than an unfamiliar individual, they exhibited enhanced BOLD signal in the NAc[7]. In prairie voles, disruption of neuromodulatory signaling within this region impairs bond formation[6], and subsequent gene expression changes contribute to bond maintenance[8,9]. However, despite substantial evidence that the NAc plays a primary role in encoding pair bonds, the neuronal dynamics underlying this process and how they change as a bond progresses remain unexplored.

We performed in vivo Ca^{2+} imaging in the NAc of freely behaving prairie voles before and after mating to gain insight into how pair bond formation and maturation are represented in the brain. Using microendoscopes, in combination with virally-delivered synapsin-driven GCaMP6f[10] (Fig 1A–E, lens placements in Fig. S1), we imaged prairie voles as they performed a 20-minute partner preference test when they were sexually naïve (Day 0), at a short-term time point following mating and cohabitation (Day 6), and again at a long-term time point following mating and cohabitation (Day 20) (Fig 1G). Because we were concerned that the brevity of the imaging sessions and attachment of the scope might weaken our ability to detect partner preference, we also performed a traditional three-hour partner preference test without the scope attached following the short- and long-term imaging sessions (Days 7 and 21). In both imaging-based and

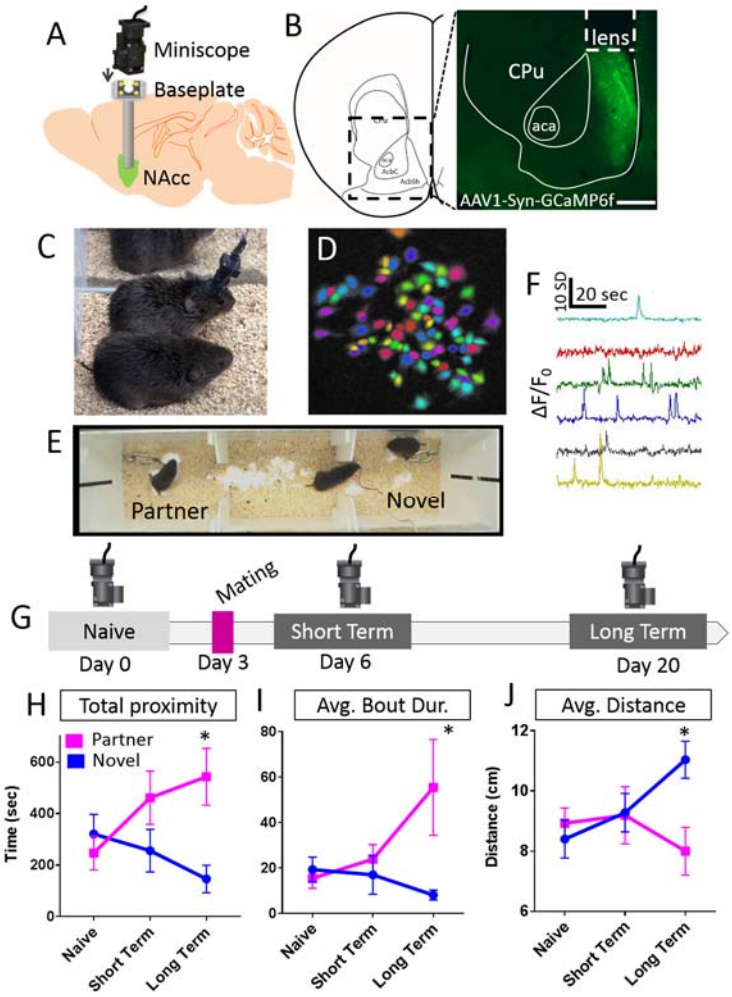

Figure 1. **Ca^{2+} imaging in monogamous prairie voles.** A) Voles were injected with AAV1-hSyn-GCaMP6f and a GRIN lens was implanted into the NAc. After recovery, a baseplate was permanently affixed to the skull to enable placement of the miniscope. B) Expression of GCaMP6f and lens insertion site. Scale bar = 500 µm. C) Two voles huddling during an imaging session. D) Putative neurons identified within the FOV of one animal. E) Imaging sessions were carried out during a 20 minute partner preference test. The test animal (center) with scope attached could freely move between two opposite sex animals tethered at either end of the apparatus. F) dF/F traces for six putative neurons. G) Experimental timecourse showing imaging sessions undertaken in sexually naïve individuals (day 0) and at short (day 6) and long term (day 20) time points after partner introduction and mating. Animals cohabited with their partner continuously beginning on day 3. H–J) Differences in social choice behavior emerged during the long-term imaging session. Test animals spent more time in proximity (<10 cm) (H), exhibited longer interaction bouts (I), and were physically closer to their mating partner (J) during the long-term imaging time point but did not exhibit differences in these metrics at the naïve or short-term imaging sessions.

traditional tests, social interaction was defined as periods in which the test animal and the tethered animal were within 10 cm of each other, a metric that correlates strongly with hand-scoring of social interaction (Fig S2B). Bond strength was assessed based on the percent of social interaction with the partner with 50% representing no preference.

We found that bonds mature with time as evidenced by emergence of a stronger partner preference with longer cohabitation and mating. As expected, sexually naïve animals did not exhibit a preference prior to mating. Three days after mating (short-term time point), we observed partner preference in the three-hour test although partner and novel-directed behavior in the imaging session was not significantly different (Fig 1H–J, Fig S2D). However, by 17 days post-mating (long-term time point), we observed strong partner preference in both the imaging session (Fig 1H–J) and in the subsequent three-hour test (Fig S2E). These data indicate that longer cohabitation leads to stronger bonds, enabling us to also ask whether bond strength is represented in patterns of NAc neuron activity.

Based on human neuroimaging studies, we hypothesized that we would observe greater overall activity while a pair bonded partner was with its partner compared with a stranger. Unexpectedly, we found that Ca^{2+} event rate across all imaged neurons did not predict whether the test animal was interacting with its partner or the novel individual after

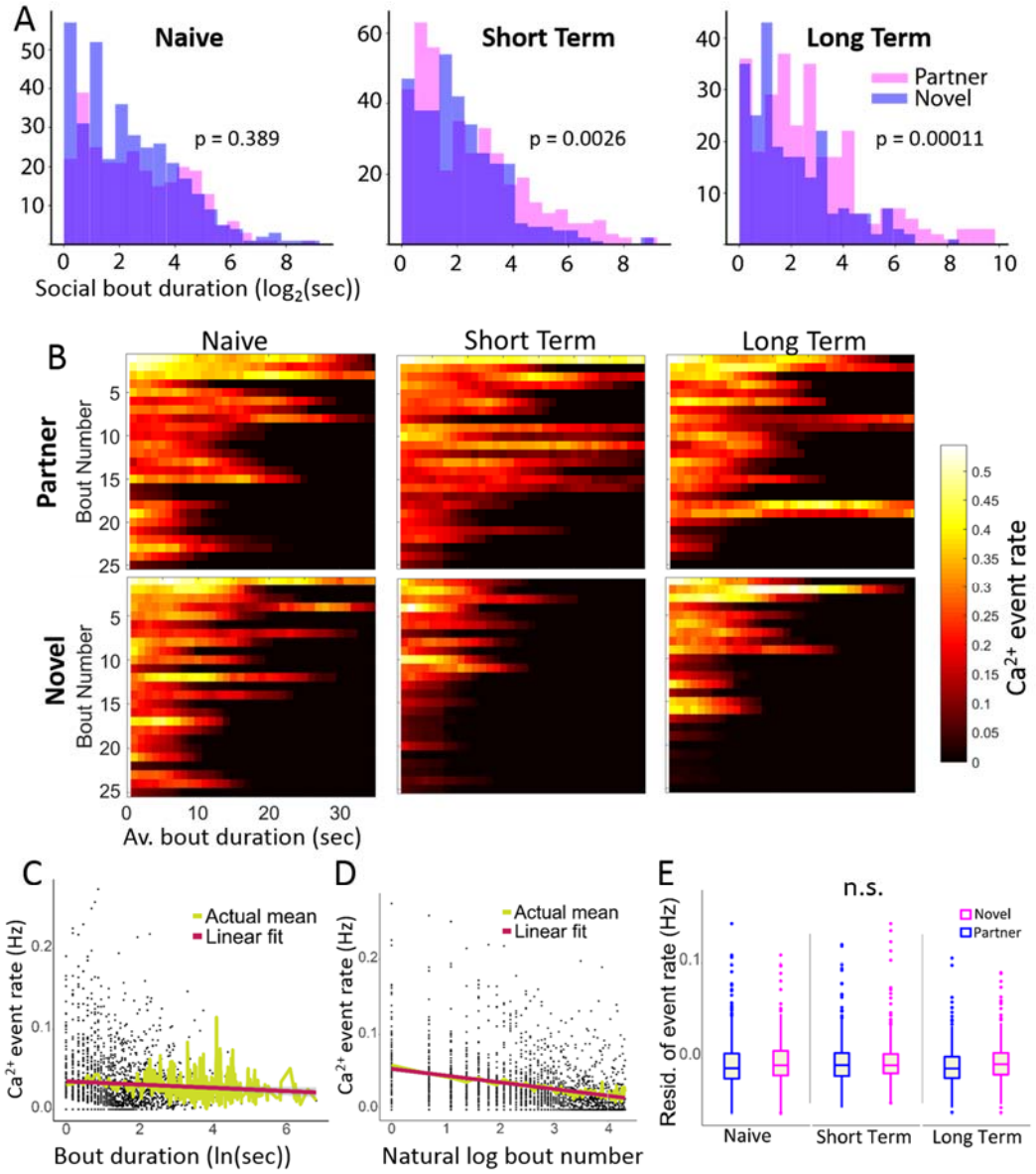

Figure 2. Intensity of Ca^{2+} activity across interaction bouts with different tethered animals pre- and post-bonding. A) Histograms showing frequency of social interaction bout lengths with partner (pink) and with novel (blue) animals. After mating/cohabitation, test animals had longer interaction bouts with their partner than with the novel individual. B) Heat plots show the average Ca^{2+} transient events (Hz) pooled across all cells and animals. Rows for social interaction bouts represent the average across all animals and were truncated at 35 seconds and 25 bouts, or when data was available for fewer than three animals. Bout length decreased across the test session and did not differ between stimulus animals during the sexually naïve imaging session. In contrast, test animals exhibited longer and more interaction bouts with their mating partner than the novel individual during the short- and long-term imaging sessions. C) Ca^{2+} activity was greater in short bouts than long bouts and corresponded with a logarithmic decrease in activity as bout length increased. D) Activity was greater during initial interaction bouts within a session and exhibited a logarithmic decrease across subsequent social interaction bouts. E) After controlling for differences in bout number and bout length, there were no significant differences in average calcium activity during partner and novel interaction. Residuals are plotted after regressing firingrate~log(duration)+log(boutnum).

controlling for differences in how the test animal interacted with each animal. We identified all periods of social interaction during the partner preference test that were at least 1 sec in duration, referring to them as social bouts. Social bout duration did not differ in sexually naïve animals investigating two novel voles, but following mating, test animals had longer social bouts with their partner than with a novel tethered animal (Fig 2A; p-values in figure). Similar to other metrics of partner preference (Fig 1H - J), the difference in partner and novel social bout duration became more significant with longer cohabitation and mating (Fig 2A). We found that average population activity rate was greater during initial social interaction bouts and at the beginning of a bout (linear regression, $p < 0.0001$ for both factors; Fig 2B, C, D, Fig S3). Thus, we used a mixed-effects model to account for differences in bout duration, bout length, and sex (see extended methods and results; Fig S3). We found that there was robust activation of the NAc during social interaction, but after accounting for differences in total number of social bouts and social bout duration, there were no differences in average activity during partner and novel interactions (Fig 2E; $p = 0.061$, $\chi^2 = 10.535$, df = 5). These findings indicate that encoding of pair bonds in the NAc does not occur via population-wide changes in activity.

We next asked whether activity in specific sub-populations of neurons might encode features of a pair bond. We used an event-triggered analysis to identify neurons whose transients corresponded with subsequent approach to or departure from the partner or novel animal, separately. For each cell, we calculated the median change in distance between the test animal and the tethered animal during a 1 second bin immediately following Ca^{2+} transients (Fig 3A). We compared the observed distance change to a null model derived from randomly shuffling each cell's transients in the partner chamber and in the novel chamber, respectively (Fig 3A). Cells with distance changes ≥ 95% of the null model were assigned as departure cells and cells with distance changes ≤ 5% as approach cells (Fig 3B). Therefore, each cell was assigned to a partner-associated category and to a novel-associated category of approach, departure, or neutral (Fig 3C, D).

We observed an expansion in the number of partner approach cells that corresponds with the behavioral changes indicative of bond maturation (Fig 3E, G). Specifically, there were no differences in partner and novel approach cells at the naïve timepoint, with differences

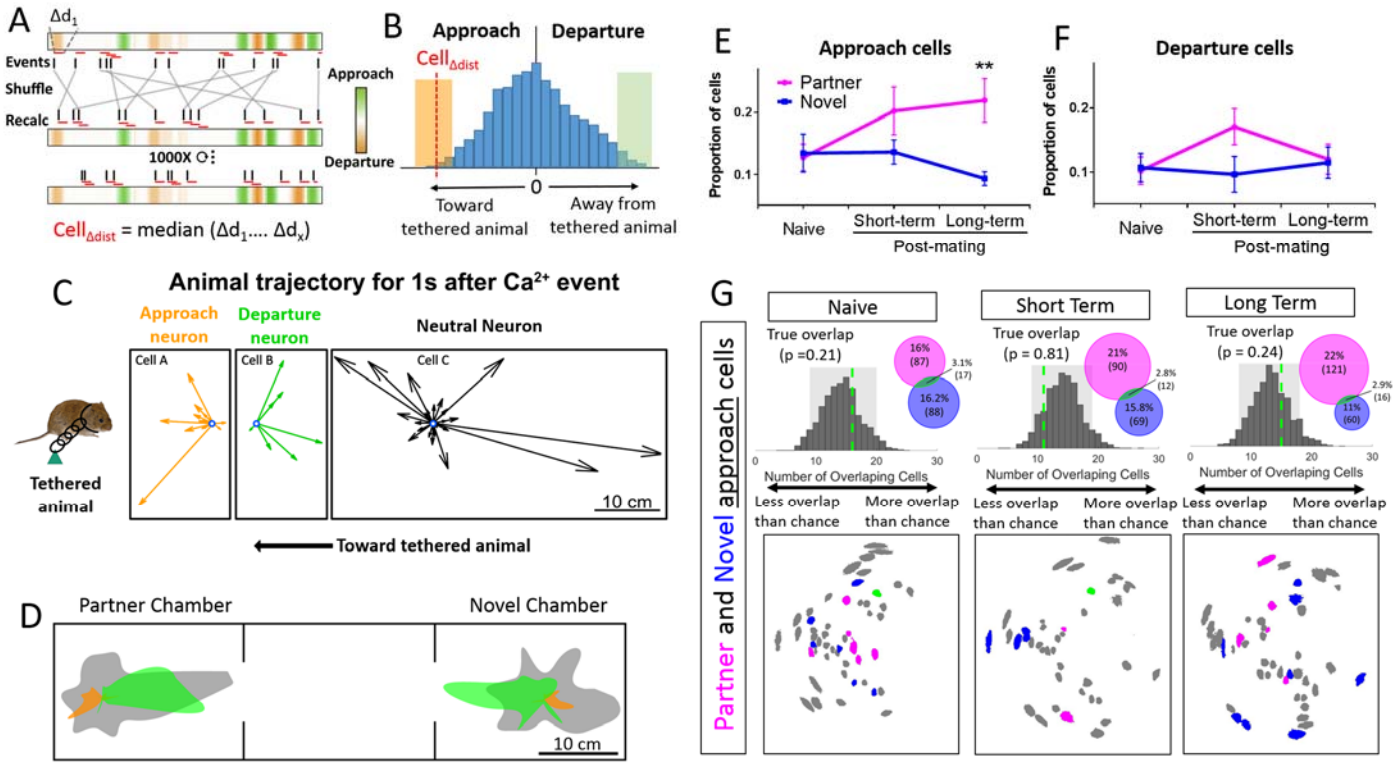

Figure 3. Approach cell ensemble increases upon bond maturation. A) We calculated the change in distance (Δd) between the test animal and the tethered animal in the 1 sec following each Ca^{2+} event (red rectangles) for a given cell when the animal was in the partner chamber or in the novel chamber, respectively. Events were then shuffled across distance data 1000 times to generate a null probability model. B) The median distance change was compared to a null probability distribution. Cells with an observed distance change ≥ 95% of the null distribution (green region) were defined as departure cells and those ≤ 5% as approach cells (orange region). C) Examples of approach, departure, and neutral neurons. Individual vectors represent change in distance from the stimulus animal during the 1 second following a Ca^{2+} transient and are plotted from the same origin. Approach vectors are shorter than departure/neutral distance vectors because the animal is bounded by the edge of the test apparatus. D) Aggregate vector maps for approach (orange), departure (green), and neutral (gray) cells with all transients normalized to the center of the partner or novel chamber. E) The proportion of partner and novel approach neurons does not differ at naïve, but significantly more partner approach neurons were identified at the long-term imaging session ($p = 0.021$). F) The proportion of partner and novel departure cells did not differ at any time point. G) Cell identity overlap between partner and novel approach cells. Chance overlap was determined by randomly assigning cell identity and calculating overlap in 1000 iterations to generate a probability distribution (gray). The observed overlap (green dotted line) between partner (pink) and novel (blue) approach cells was not greater than would be expected by chance. Cell identity plotted for each time point for one animal (pink = partner approach, blue = novel approach, green = both.)

emerging post-mating and becoming significant by the long-term time point. The expansion of the partner approach cell ensemble indicates that that bond maturation may result in changes in how partner approach and novel approach are represented in the NAc (Fig 3E; main effect of partner identity, $F_{1, 11} = 7.252$; $p = 0.021$). In contrast, no differences were observed in partner and novel *departure* cells at any time point even though these cells were identified in the same permutation analysis (Fig 3F; main effect of partner identity, $F_{1,11} = 1.106$, $p = 0.316$; interaction $F_{2,22} = 1.91$, $p = 0.172$). Thus, differences in approach ensembles are unlikely due to an unanticipated variable because such a variable would equally affect identification of approach and departure cells. In addition, these cells were not predictive of direction of travel relative to the apparatus ($p > 0.05$; further details in supplement).

Are partner and novel approach cells distinct populations? We found that partner and novel approach neurons did not overlap more than would be expected by chance (Fig 3G; naïve, $p = 0.23$; short term, $p = 0.79$; long term, $p = 0.25$), suggesting that partner and novel approach are independently represented in separate ensembles. Somewhat surprisingly, partner and novel departure cells overlapped more than would be expected by chance (Fig S4; naïve, $p = 0.004$; short term, $p = 0.014$; long term, $p = 0.019$). A possible interpretation of these findings is that approaching the wrong animal could have deleterious consequences (e.g. from aggression). Distinct approach ensembles may be critical for identifying and deciding to approach a specific individual, while that level of specificity is not necessary when departing from a social interaction.

Finally, we found that the majority of approach and departure cells exhibited transients prior to rather than during social approach or departure, indicating that these cells may directly modulate the decision to approach or leave the tethered animal. The method we used to identify approach and departure cells did not distinguish between cells whose events preceded transition to approach or departure versus those where events occurred during ongoing approach/departure. To determine whether approach cell transients primarily occur during or prior to approach, we carried out the same permutation analysis but calculated the change in distance between test and stimulus animal for the one second ***prior*** to a Ca^{2+} event to identify cells in which transients consistently occurred after the test animal had already initiated approach. We found that on average 26.3% (range: 17.4–30.7%) of approach cells and 38.8% (range: 25–47.4%) of departure cells represented cells whose Ca^{2+} events occurred while the animal was already approaching or departing (supplemental table 2). For the remaining cells, approach began after the transient, suggesting a potential behavioral transition.

Pair bonding changes the brain, and our experiments have now identified a neuronal substrate contributing to these changes. Somewhat surprisingly, we find that partner-associated differences in overall NAc Ca^{2+} activity are not evident in pair bonding in prairie voles. Instead, specific features of pair bonding, such as the preferential desire to approach a partner rather than a stranger, may be encoded in specific neuronal ensembles. Partner approach neurons exhibit a number of features that make them an ideal candidate for encoding key aspects of a pair bond, such as the decision to approach a pair-bonded partner. First, the expansion of the partner approach ensemble closely parallels the emergence of partner preference. Second, the partner and novel approach ensembles are non-overlapping, which would be expected if these cells contain information about the specific animal being approached. Finally, most of these cells have Ca^{2+} transients prior to rather than during ongoing approach, suggesting they contribute to the decision to approach a particular animal. In sum, we have identified a neuronal ensemble whose activity may drive partner reunion and whose expansion reflects bond maturation, providing a critical neuronal substrate for monogamy.

Author Contributions and Notes

Z.R.D., M.A.K. and J.C.J. designed research, J.L.S and E.K. performed research, E.V., D.S.W.P., E.S., and R.C. analyzed data; and Z.R.D. wrote the paper.
The authors declare no conflict of interest.
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