The Effects of Gradual Transitions Between Neutral and Dangerous Contexts on ventral CA1 Pyramidal Neurons in Mice

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Abstract

An organism's survival depends upon the ability to discriminate between safe and dangerous environments. Spatial memory is thought to critically depend on the hippocampus (HPC) as it contains "place cells" that are maximally active when an animal occupies a discrete spatial location (Moser et al., 2018). Together, hundreds of place cells form a cognitive map that is necessary for navigation and contextual memory (Moser et al., 2018). However, in pathologies such as post traumatic stress disorder (PTSD), an inability to distinguish between safe and dangerous environments can induce persistent re-experiencing and abrupt flashbacks (Jovanovic et al., 2011). To better understand the mechanisms of a complex psychiatric disorder like PTSD, we explored how a rodent (mouse) model reacts to slow transitions between a fear-conditioned context and a neutral context. *In vivo* calcium imaging was used to record place cells in the ventral CA1 (vCA1) using fiber photometry. Two contexts were represented within the confines of a 90cm diameter LED monitor arena and differentiated based on audio and visual output. One context was associated with fear via the administration of foot shocks. Based on behavioral freezing differences between contexts after training, it was determined that the mice successfully discriminated the environments. Calcium changes in pyramidal vCA1 neurons support the claims that vCA1 neurons are implicated in contextual fear discrimination and that their activity is correlated with behavioral fear expression (freezing). However, there was no evidence to suggest that vCA1 activity fundamentally reacts differently to entering a dangerous context versus a neutral context.

Significance Statement

Post traumatic stress disorder (PTSD) is linked with persistent re-experiencing and abrupt flashbacks which may be due to the brain's inability to separate dangerous from safe environments (Jovanovic et al., 2011). It has been shown that the hippocampus is highly implicated in this disorder (Fenster et al., 2018). Thus, there is evidence to suggest that an examination of groups of neurons within the ventral hippocampus during contextual fear discrimination will provide valuable insight into the working mechanisms behind the abrupt "re-experiencing" burdened by those suffering from PTSD.

Introduction

An organism's survival depends upon the ability to discriminate between safe and dangerous environments. However, in certain pathologies such as post traumatic stress disorder (PTSD), an inability to distinguish between safe and dangerous environments can induce persistent re-experiencing (Jovanovic et al., 2018). To better understand the mechanisms of a complex psychiatric disorder like PTSD, we explored how a rodent (mouse) model reacts to slow transitions between a fear-conditioned context and a neutral context.

The hippocampus (HPC) is known to have essential roles in spatial memory, navigation, and adaptive context-dependent fear responses (Moser et al., 2018). It contains "place cells" that are maximally active when an animal occupies a discrete spatial location (Moser et al., 2018). Together, hundreds of place cells form a cognitive map that is necessary for navigation and contextual memory (Moser et al., 2018). The HPC thus understandably plays a key role in contextual fear conditioning and the remembering of pertinent features of an environment (Frankland et al., 1998). However, the defined circuits and place cell populations encoding

dangerous and safe contexts are not fully characterized. Interestingly, ventral CA1 neurons (vCA1) project directly to the amygdala (AG), a region that is crucial for the expression of learned fear responses and fear conditioning (Sanders et al., 2003; Whalen et al., 1998). vCA1 also connects to the medial prefrontal cortex (mPFC), a structure that has recently been shown to have an important role in fear discrimination (Dejean et al., 2015; Ciocchi et al., 2015). Thus, recording from pyramidal neurons in vCA1 presents an interesting way to analyze the processes behind contextual fear discrimination.

In the current study, we developed a new fear discrimination paradigm where different contexts are represented within the confines of a large 90cm diameter circular LED screen (Figure 1.E). They are differentiated by the visual image the LED screen displays and by the audio output of ground speakers. This paradigm enables context transitions to be controlled very easily without the need to transport the mouse. Instead, the mouse can be 'teleported' from one context to another. Moreover, transitions can be slowed down as one context steadily morphs into the other. Using multi-channel fiber photometry, a powerful tool that records global neuronal activity from a population of neurons with a high temporal resolution (Guo et al., 2015), vCA1 pyramidal neurons were recorded as contexts slowly morphed into each other and features of the new environment became more and more apparent. Since one context was associated with fear via the administration of foot shocks, the circuitry behind contextual fear discrimination was explored.

Considering these functional and anatomical findings, we hypothesize that vCA1 projections are involved in the process of contextual fear discrimination. This involvement should be recognizable by changes in global neuronal activity during gradual transitions from context to

context. In order to test that the mice differentiate between the two contexts and associate one with fear, there should be contrasting freezing levels in the two contexts after training.

Materials and Methods

Experimental Animals

All mice used were male, type C57BL6/j and aged 8-10 weeks prior to surgery (Charles River Laboratory). They were individually housed, given water and food *ad libitum* and were maintained under a 12-hour light and dark cycle. Mice were experimented during the light cycle. All procedures were approved by the Facility Animal Care Committee (FACC) of McGill University.

Surgical Procedures

Mice were first anesthetized with isoflurane (4% during induction and 2% during the surgery) in O2. Then, mice were placed in a stereotaxic frame and body temperature was maintained with a heating pad. Carprofen (5mg/kg) and saline solution (0.9 mg/kg) were subcutaneously injected and eye-protective cream was applied. After fur removal on the dorsal portion of the head, iodine solution (Betadine) was used to clear the skin surface. A sterile scissor was used to then make an incision. The head level was adjusted by placing bregma and lambda on the same horizontal plane. Craniotomies were performed in the left hemispheric vCA1: -3.28 AP, -3.15 ML, -3.4 DV (in relation to bregma, Paxinos and Franklin, 2nd edition, 2001).

To record pyramidal vCA1 neurons, AAV5-CaMKII-GCaMP6f-WPRE-SV40 (4.11 x 1013 vp/ml, obtained from Penn Vector Core, University of Pennsylvania, Philadelphia, PA) virus solution was loaded into a pulled borosilicate glass capillary and unilaterally injected into the vCA1 (same coordinates as above) with a Nanoject II (Drummond Scientific Company). Volumes

of 0.5 μL virus solution were injected. Then, the pipette was left in place for 10 minutes following the injection before being retracted slowly.

Around two weeks after AAV injection, an optic fiber cannula (400 μm diameter, Doric Lenses) was implanted into vCA1 (same coordinates). Optic fibers were fixed to the skull using dental cement and then finished with black nail polish. After surgery, mice recovered on a heat pad and then returned to their home cage. After 2 to 3 weeks, experiments commenced.

Behavioral Apparatus

The apparatus was composed of a large cylindrical LED screen (77 cm high, 90 cm diameter) that projected a visual display (Figure 1.E). The floor contained stainless steel rods that were wired to a shock source and a solid state grid scrambler for the delivery of footshocks. Auditory stimuli were delivered via ground speakers that were below the floor. A 4m long fiber optic patch-cord (Doric Lenses) was connected to the chronically implanted optic fiber and was suspended above the behavioral testing environment to allow animals to move freely during context presentations. A Logitech c930e camera was mounted on the ceiling of the arena to track the mouse (Figure 1.B). To coordinate the acquisition of behavior and physiology, Bonsai (Lopes et al., 2015) programs acted as the central commander, controlling the camera, shock generator, photometry acquisition, ground speakers and visual display of the screen. This co-ordination enabled the direct comparison of camera frames (and mouse location) to neuronal activity.

Day 1 and 2 (Pre-exposure and Fear Conditioning)

In the week leading up to the experiment, mice were handled for about 5-10 minutes on 3 occasions, which consisted of picking up the mice and simulating the process of attaching the fiber

photometry system. Day 1 and day 2 were conducted exactly the same; in the morning (starting around 9:00 a.m.) the mice were exposed to the neutral context A for 12 minutes. Then, 6 hours later (at around 3:00 p.m.), they were exposed to context B for 12 minutes which was accompanied by foot shocks. This 6-hour delay was incorporated in order to prevent generalization of the two contexts. Figure 1.A concisely shows the procedure for day 1 and 2.

When exposed to context A in the morning, mice were transported in their home cage to the experimentation room. In the room, lights were off (except for a small lamp) and the mice were quickly attached to the photometry acquisition system. Then, they were placed in the LED arena with context A already being portrayed. Context A had a darker visual display and had a constant 10Hz tone being played by the ground speakers.

When exposed to context B, mice were transported in a small circular box (Figure 1.D) to the experimentation room. In the room, lights were on and the mice were quickly attached to the photometry acquisition system. Then, they were placed in the LED arena with context B already being displayed. Context B had a lighter visual display and no sound playing from the ground speakers. The mice subsequently experienced 6 foot shocks (1s each at an intensity of 0.8 mA with an inter-trial interval of 50-100 s, starting after 4 minutes of exploration). The first shock occurred after 4 minutes for the mice to become accustomed to the environment and register the relevant features before being conditioned.

Day 3 and 4 (Testing fear discrimination and transitions between contexts)

Day 3 and 4 were conducted very similarly to each other; at around 12:00 a.m. (time chosen in order for time of day not to influence the outcome), mice were tested in configuration BABABA on day 3 and ABABAB on day 4. Mice thus underwent a suite of six contexts presented for 110

seconds each. Between each context, mice were exposed to a 16 second gradual transition period called an "AB" transition from context A to B and a "BA" transition in the opposite direction. On day 3, the mice experienced context B first and were transported with the black box and the room lights on. On day 4, the mice experienced context A first and were transported in their home cages with the room lights were off. Figure 1.C concisely shows the procedure for day 3 and 4.

Each mouse thus experienced 3 BA transitions and 2 AB transitions on day 3 and conversely experienced 3 AB and 2 BA transitions on day 4. In all, each mouse experienced 5 BA transitions and 5 AB transitions. Days 1,2,3 and 4 all happened consecutively in time.

In order to carry out the 'transition periods', the visual and audio signatures were gradually changed from the characteristic features of one context to the other. For the visual display, the two context's images were overlaid and their transparency values linearly changed every second over the 16 second period to go from either 100% to 0% or vice versa. For the audio signature, the sound coming from the ground speakers gradually went from 10Hz to 0Hz or vice versa. At the 8 second mark of the transition, the context represented could be considered the exact average of context A and B.

Photometry Acquisition

The photometry system used two excitation LEDs: a 405 nm LED for calcium-independent fluorescence and a 465 nm LED for calcium-dependant GCaMP6f fluorescence. The excitation light was modulated at 211 Hz and 531 Hz respectively. The excitation light was coupled into a low auto-fluorescence 400μm patch cable with a high numerical aperture coupler/collimator. Both LEDs were driven at 200 mA, producing power ranging from 20-40μW at the tip of the patch cable (Power meter, Thorlabs). The far tip of the patch cord was connected to a metal optical ferrule/fiber (1.25 mm diameter ferrule, 400 μm diameter fiber core) with an opaque zirconia mating sleeve.

405 and 465 nm lights excite GCaMP6f infected neurons upon calcium influx. Then, GCaMP6f emission signals were collected in a femtowatt photoreceiver using a focusing lens. Photoreceiver signals were relayed to the Doric Photometry Console for demodulation into two signals corresponding to each LED excitation wavelength based on its modulation frequency. These outputs were digitized at a sampling rate of 12,000 Hz and decimated by a factor of 50.

The 405nm wavelength served as a control and represents GCaMP fluorescence that isn't calcium activated while the 465nm wavelength records the GCaMP activity of interest – relating to neuronal activation. Both the 465nm and 405nm wavelength recordings underwent a post-hoc least mean squares analysis to convert the traces to units of $\Delta F/F_0$. Then, the 405nm wavelength is subtracted from the 465nm wavelength in order to isolate the activity of interest. This process of subtraction helps alleviate concerns over noise and external phenomena affecting signal since only calcium dependent GCaMP remains. Moreover, it standardizes signals from different time points and days which allows for their comparison.

Locomotion

Locomotion behavior was recorded using the Logitech c930e camera at a rate of 30FPS. Using HSV (Hue-Saturation-Value) thresholds, the mouse was isolated from its surroundings and its centroid position (location) was recorded at every frame. Centroid positions were smoothed post-hoc with a sliding window of 14 frames. Centroid position null values (when the tracking failed) were linearly interpolated. Velocity at any given time point was determined by the change in centroid position divided by the change in time over the two camera frames. Velocity values were also smoothed post-hoc with a sliding window of 2 frames. A freezing epoch was determined to be when the mouse had a velocity strictly under 1.5cm/s for at least 2 seconds. This criteria most accurately described when the mouse had no visible movements and adopted typical freezing

behavior. Automating the tracking removed potential biases from the analysis and ensured standard criteria for every run.

Experimental Design and Statistical Analyses

The current study was originally supposed to incorporate 10 mice. However, one had to be sacrificed due to a failure with the head stage after surgery so only 9 mice were tested. For every 110s exposure to either context A or B on days 3 and 4, the mouse's freezing percentage was calculated according to the formula: #seconds freezing / #seconds in context * 100%. One-way ANOVAs were performed to test the significance of context on freezing percentages.

A Discrimination Index (DI) was constructed to test the behavioral freezing of each mouse individually (Table 1). The difference in freezing before and after every AB transition (expected to be positive) and before and after every BA transition (expected to be negative) were recorded. The BA average was subtracted from the AB average for every mouse. This number was divided by the total average freezing percentage of the mouse (during days 3 and 4 combined) in order to control for the fact that some mice simply froze more. A higher DI signifies better behavioral expression of contextual fear discrimination.

As a test for the net effect of a transition period on vCA1 calcium activity, every 16 second transition period's photometry data was linearly fitted using the "polyfit" (Matlab 2018b) function and the slope of that fit was retrieved. One-way ANOVAs were performed to detect differences in slope between AB and BA transitions for each mouse.

Linear correlation coefficients throughout the study were calculated with the following formula: $$Correl\,(X,Y) = \frac{n(\sum xy) - (\sum x)(\sum y)}{\sqrt{[n\sum x^2 - (\sum x)^2][n\sum y^2 - (\sum y)^2]}}$$

These coefficients were used to test the correlation between p-values of the aforementioned slope ANOVAs and the discrimination index of each mouse.

Each individual transition can be characterized as having a calcium activity slope (as mentioned) as well as a difference in behavioral freezing due to that change in context (freezing % after transition – freezing % before transition). These 2 values were correlated using the above formula for all the AB transitions and BA transitions of each mouse.

For every transition, the 1 second window with the minimum average signal and the 1 second window with the maximum average signal was calculated. The amount of time between the middle of these 2 one second windows is considered the 'change duration' of the vCA1 activity for that transition. Whichever time came first is considered the 'start' of the vCA1 change and can loosely be considered when vCA1 recognized context was changing. One-way ANOVAs were performed to test the differences between these 2 values for BA transitions vs. AB transitions.

Results

Behavioral Expression of Contextual Fear Discrimination

During days 1 and 2, context B was accompanied by foot shocks while A was not. In order to test that the mice could distinguish the environments and were more afraid of context B, behavioral freezing was observed in the different contexts during days 3 and 4. Each mouse experienced 6 110 second exposures to context A as well as context B. The percent of time spent freezing during every epoch of context A and B was calculated (Figure 2). Using a one-way ANOVA, a significant main effect of context on freezing percentage was found with mice freezing significantly less in context A ($\mu = 39.75\%$) than context B ($\mu = 53.76\%$), $F(1,113) = 9.35$, $p = 0.00323$ (Figure 2.D). This supports the fact that the mice discriminated between the contexts and were more afraid (i.e. froze more to) the context in which they experienced foot shocks.

Net vCA1 change during transition period (AB vs. BA)

As was mentioned, each mouse encountered 5 'BA' transitions (from context B to A) and 5 'AB' transitions (from context A to B). See Figure 3. For each BA and AB transition, a linear fit line was calculated over the 16 second transition period where the slope of that line was observed. A positive slope suggests that from the beginning of the transition to the end, global calcium activity increased. For each mouse, a one-way ANOVA was performed on the slopes of the 5 BA transitions compared to the 5 AB transitions. For 4 of the 9 mice, there was a significant effect of transition direction on slope (Figure 4). 1FP6 showed increased neuronal activity from the AB direction ($\mu = 0.14$) compared to the BA direction ($\mu = -0.33$), $F(1,9) = 6.4$, $p = 0.0354$. 3FP6 also showed increased neuronal activity from the AB direction ($\mu = 0.028$) compared to the BA direction ($\mu = -0.072$), $F(1,9) = 7.89$, $p = 0.0229$. Oppositely, 2FP6 showed increased neuronal activity from the BA direction ($\mu = 0.86$) compared to the AB direction ($\mu = -0.37$), $F(1,9) = 7.44$, $p = 0.0259$. Last, 27FP4 also showed increased neuronal activity from the BA direction ($\mu = 0.23$) compared to the AB direction ($\mu = -0.11$), $F(1,9) = 21.15$, $p = 0.0018$. The other 5 mice did not show significant differences in slope during AB transitions compared to BA transitions. Moreover, a one-way ANOVA showed no significant difference between the overall slope of AB transitions ($\mu = 0.083$) and BA transitions ($\mu = 0.0026$) across mice, $F(1,17) = 0.325$, $p = 0.576$.

Correlation between discrimination index and net vCA1 change during transitions

For every aforementioned slope analysis, the ANOVA yielded a p-value which represents the strength of significance. A smaller p-value signifies that the difference between the BA and AB transitions (if any) were more significant. When correlating these p-values with each mouse's discrimination index, the correlation coefficient found was $\rho = 0.019$ which is not significant ($|\rho|$

> 0.70). See figure 5. This means that the discrimination index of a mouse did not predict whether an AB transition would cause a different net vCA1 change than a BA transition. In other words, overall behavioral discrimination of a mouse did not predict its overall vCA1 discrimination.

Correlation of net vCA1 activity during a transition and behavioral freezing after the transition

To recap, each 16s transition period induced a certain amount of calcium change in vCA1. This neuronal activity was fitted to a linear line with a given slope. Each 16s transition period was sandwiched between 110s epochs of context A and B (Figure 1.C). The percent freezing in the context after the transition was subtracted from the percent freezing in the context before the transition. Then, this difference was correlated to the vCA1 slope of that transition (AB and BA transitions examined separately). See Figure 6. In 5 of the 9 mice, there was a significant correlation between one of the transition directions' net vCA1 change and its freezing changes – meaning that a large net calcium change *during* the transition had a strong correlation with the freezing *after* that transition. 1FP6 showed a correlation coefficient $\rho = 0.96$ in the BA direction, 4FP6 had a $\rho = -0.74$ in the AB direction, 6FP6 had a $\rho = 0.88$ in the AB direction, 25FP4 had a $\rho = -0.79$ in the AB direction, and 27FP4 had an $\rho = 0.87$ in the BA direction. Across all mice, the average AB transition had a $\rho = 0.50$ and the average BA transition had a $\rho = 0.48$. None of the mice had significant correlations in both the AB and BA direction.

Time component of vCA1 change due to transitions

During the 16 second transition periods, there is a 1 second window with the highest average calcium activity and a 1 second window with the lowest average calcium activity. The speed of neuronal change was observed by comparing the time between the middle of these one second windows and is referred to as the 'change duration'. vCA1 activity could have increased

or decreased due to the transition. See figure 7.A. There was no significant difference in change duration between the BA and AB transitions across all the mice according to a one-way ANOVA $F(1,89) = 0.046$, $p = 0.83$. The mean change duration was 7.44 seconds.

The start of these durations was also recorded and is referred to as the 'start' of the vCA1 change. This could be considered when vCA1 recognized a change was happening. See figure 7.B. There was no significant difference in start times between BA and AB transitions $F(1,89) = 0.098$, $p = 0.92$. The overall average 'start' time was 4.47 seconds after the transition commenced.

Discussion

To recap, mice were exposed to 2 contexts (A and B), one of which (B) was accompanied by foot shocks during the first 2 days of training. Then, mice experienced five 16 second gradual transitions from context A to B (AB) as well as from context B to A (BA) over the course of 2 consecutive testing days. Neuronal activity was recorded from vCA1 pyramidal (projection) neurons.

It appears as though the fear conditioning paradigm worked in inducing discrimination due to the significantly different freezing percentages in contexts A and B (figure 2.D). Contextual fear discrimination was thus accomplished without changing the shape of the context (Leutgeb et al., 2014), cues (Chess et al., 2019), or physically transporting the mice into a new location (Frankland et al., 1998) as has been shown to be effective in the literature. However, a previous study was able to induce contextual fear discrimination by changing features (smell, visual output, audio tone) of a testing chamber (Rozeske et al., 2018) which is similar to the paradigm used in the current study. It's worth noting that the mice still froze a certain amount in context A (figure 1.D). Although context A was associated with some level of fear, all that was important for the current study was contrasting levels of freezing between the two contexts.

To test whether vCA1 activity increased or decreased from the start to end of a transition period, a linear line was fitted to each transition's vCA1 activity and the slope was recorded. Slopes are informative about the net change in neuronal activity but they assume a linear increase/decrease. However, there is qualitative evidence to suggest parabolic calcium signatures as well (Figures 3.A, 3.E, 3.F). The slopes of AB transitions were compared to the slopes of BA transitions for each mouse. One of the most interesting findings of this study was the fact that for 4 of the 9 mice, there was a significant difference in net vCA1 change (slope) between the 5 BA transitions and 5 AB transitions (Figure 4). This supports the notion that there are neurons in vCA1 who are preferentially activated when the mouse's environment changes to a specific context. However, it is curious that this significant effect was only replicated for 4 of the 9 mice. One theory is that for the other mice, they simply failed to discriminate the contexts. However, it doesn't appear as though this is true since there was no correlation between each mouse's discrimination index and the p-value of their slope analyses (Figure 5). A theory that may explain the variability between mice is the fact that there are many different types of pyramidal neurons in vCA1 (Ciocchi et al., 2015). Pyramidal neurons in vCA1 have been shown to project to the mPFC, AG, nucleus accumbens etc… (Ciocchi et al., 2015). The optic fiber is only sensitive to a certain population of vCA1 neurons. This data may be the consequence of optic fibers in different mice being next to different types of neuronal populations/cells that behave differently to transitions.

Another interesting observation about the net change in vCA1 activity (slopes) during transitions was that the direction of transition did not always induce the same neuronal effect across mice. BA transitions led to more neuronal activity in some mice while AB transitions led to more neuronal activity in other mice (Figures 3 and 4). Like the previous observation, this effect may be due to the population of neurons around the optic fiber. The hippocampus has place cells that

are modulated by both location and environment (O'Keefe et al., 1998). If optic fibers in different mice surrounded neuronal populations geared more to one of the contexts, it would explain why the direction of change was not conserved. Although all the optic fibers were in the same location with relation to Bregma, there is no relation between the anatomical location of cells in the hippocampus and their place fields (O'Keefe et al., 1998). So, this data doesn't provide evidence that vCA1 neurons fundamentally behave differently when entering a dangerous versus safe context, since both directions induced similar changes to vCA1 activity in different mice. However, the fact that the direction of change in vCA1 activity was different for different mice supports the notion that there are cells in vCA1 that are preferentially activated when entering a specific context (whether it be dangerous or neutral).

It is one thing for vCA1 projection neurons to react to entering different contexts, but it is another for that activity to be related to behavior. The amount of neuronal change *during* a transition often correlated with the behavioral freezing change *after* that transition. This effect was found in 5 of the 9 mice in one of either the AB or BA direction (Figure 6). Interestingly, a mouse never had significance in both directions. This supports the claim that there are pyramidal cells who's firing is specific to entering specific contexts, otherwise one would expect to see significance in both directions. Moreover, this data hints that there is a correlation between behavioral expression of fear and neuronal activity in vCA1. Perhaps the ventral hippocampus is not only implicated in the recognition of different contexts, but has a role in encoding the levels of fear associated with them.

The start and duration of vCA1 changes during transitions were recorded. The biggest problem with this analysis is that there is no guarantee that the minimum/maximum vCA1 activity is really the start/end time of the reaction to context change. There are various explanations for

why a signal might be very low/high, even as simple as location. Nevertheless, it was found that the average duration of vCA1 change lasted 7.44 seconds and the average start of this change occurred 4.47 seconds after the start of the transition. There were no significant differences in these variables between AB and BA transitions (Figure 7). From an evolutionary perspective, it might have made sense for AB transitions to be recognized faster as it is more important to recognize dangerous environments quickly. However, this was not observed. The data did support the idea that vCA1 place cells can recognize a context with limited features of the environment present. The 'start' of change on average occurred after 4.47 seconds which is well before even half of the features of the new environment had been present. The fact that the duration of change took around 7 seconds is also interesting. This data goes against the notion that there is an all-or-none abrupt change in vCA1 place cells and hints at gradual changes in population activity. Perhaps as more and more features of the new environment accumulated, more cells became active (that might be preferentially activated to specific features); or, perhaps neurons geared to one context adopted intermediary states as the environment slowly shifted. This observation may have not been possible if the context changes were instantaneous and all the features of the new context were revealed at once.

Future Experimentation

Slight Changes to Current Study

The current study used transition periods of 16 seconds. It would be interesting to test the effect of transition periods with varying lengths. For example, the more gradual the transition, the longer it might take for vCA1 activity to change. Studies centered around the time course of the vCA1 change could help answer questions about whether or not vCA1 neurons change abruptly

or gradually when context changes. As was mentioned, this research is highly implicated in psychological disorders such as PTSD. PTSD lasts much longer than a couple of days and so testing contextual fear discrimination and vCA1 activity weeks after training might also be worthwhile.

Contrasting vCA1 projection neurons that target the amygdala and pMFC

In the current study, pyramidal neurons in vCA1 are targeted but it remains unclear where exactly they are projecting. As was mentioned, this could be the cause of some of the variation that was found across mice as the optic fiber may have been next to neurons projecting to the amygdala, mPFC, etc…. A retrogradely transmitted virus encoding CRE injected into the AG coupled with CRE-dependent GCAMP in vCA1 would ensure only neurons projecting to the AG would be recorded. It's possible that these cells behave very differently than those that project to the mPFC when context is changed. A similar technique could be used in order to observe only vCA1 neurons that project to the mPFC. The contrast between different types of vCA1 projection neurons might provide valuable insight into the mechanisms of contextual fear discrimination.

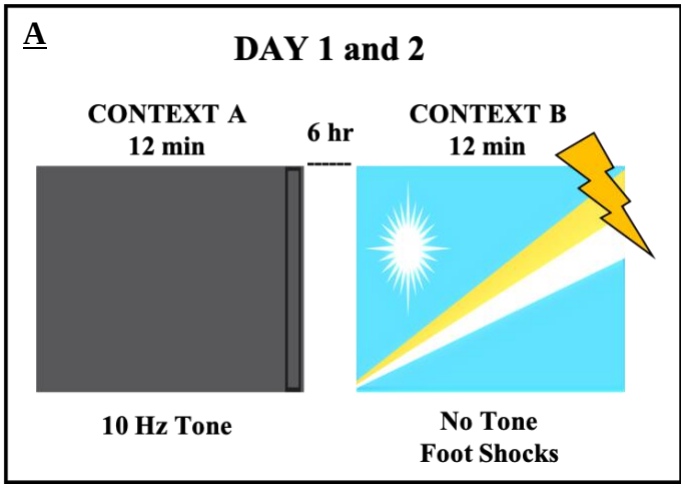
Miniscope Recordings

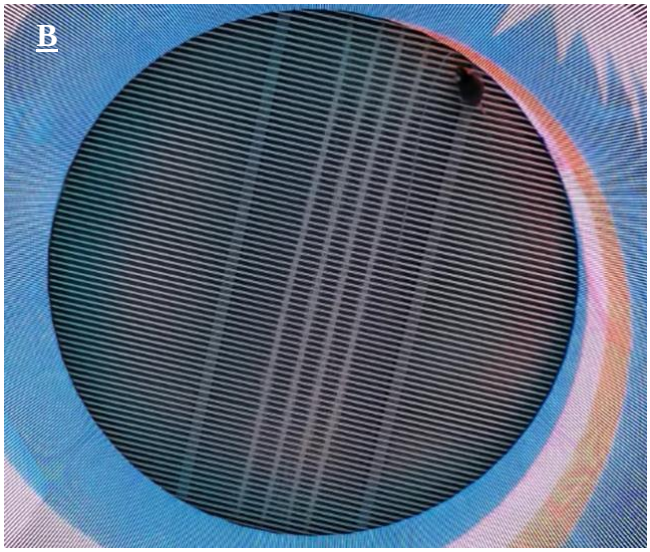
Using a miniscope to record vCA1 place cells would be a great way to further expand on this project. The miniscope presents a major advantage which is the analysis of individual place cells with spatial and temporal resolution. While photometry is powerful, only global activity can be detected. This could remain unchanged even though individuals neurons are undergoing drastic remodelling. Miniscope analysis could explain whether vCA1 neurons adopt intermediary states during a transition or whether there is a slow rise in the number of neurons turning on/off. This observation would expand on the analysis of net vCA1 change during transitions. It would also be

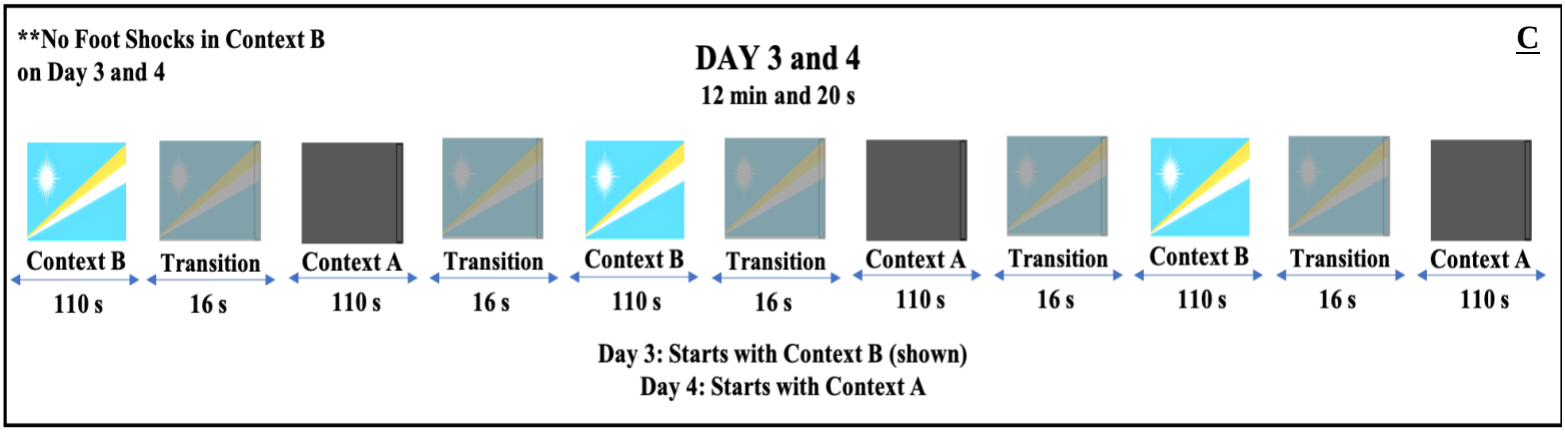
interesting to examine how neuronal place fields change in a context that is half A and half B (such as during the 8 second mark of the transition). Perhaps the dangerous context would be dominantly represented by vCA1 neurons since evolutionarily, it might be more important that it is recognized. Or, perhaps half the neurons in vCA1 would represent context A while the other half represent context B.

In all, there's a variety of future experiments that could advance this research. The paradigm used by the current study is flexible and enables easy context representation. Based on the literature, there is clearly evidence to suggest that the hippocampus' interactions with the mPFC and amygdala are crucial for contextual fear discrimination and that the circuitry is far from being sufficiently understood.

Figures







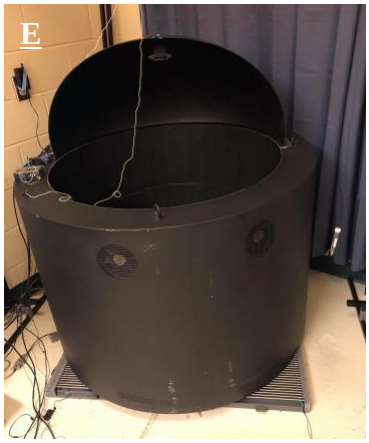


Figure 1: Paradigm set up. 1.A shows the procedure for days 1 and 2. 1.B shows a view from the behavioral camera during an epoch of context B. 1.C shows the procedure for days 3 and 4. 1.D shows the black box sometimes used to transport the mouse. 1.E shows the LED monitor arena.

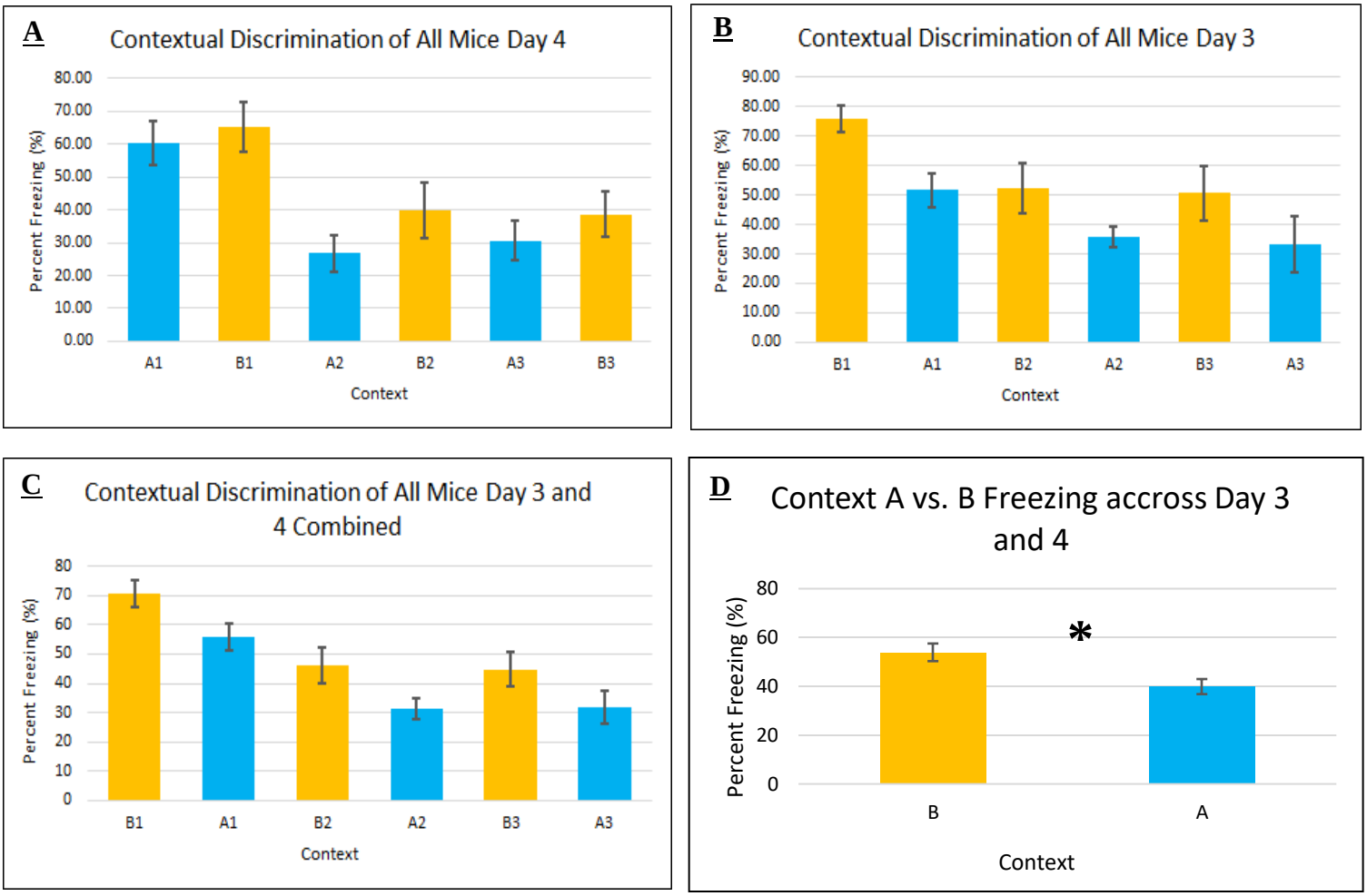
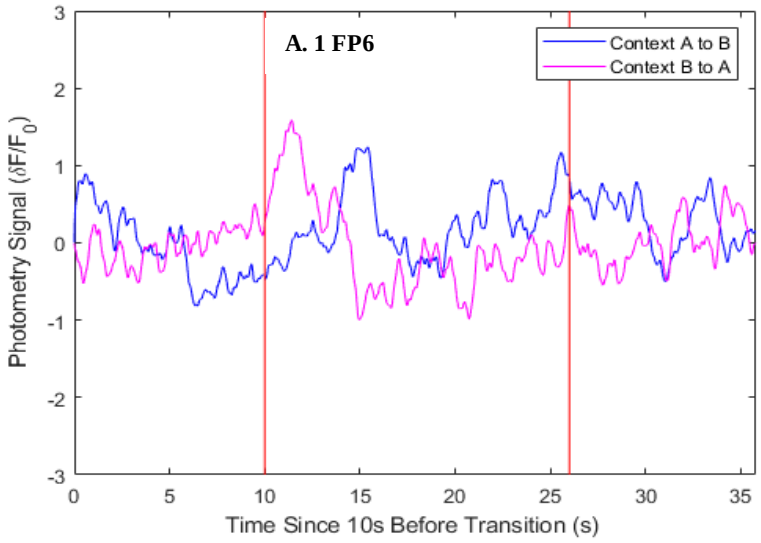
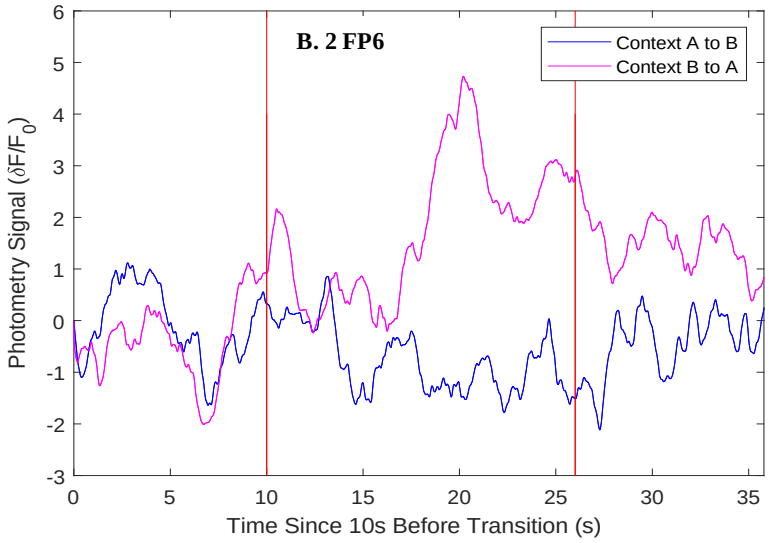
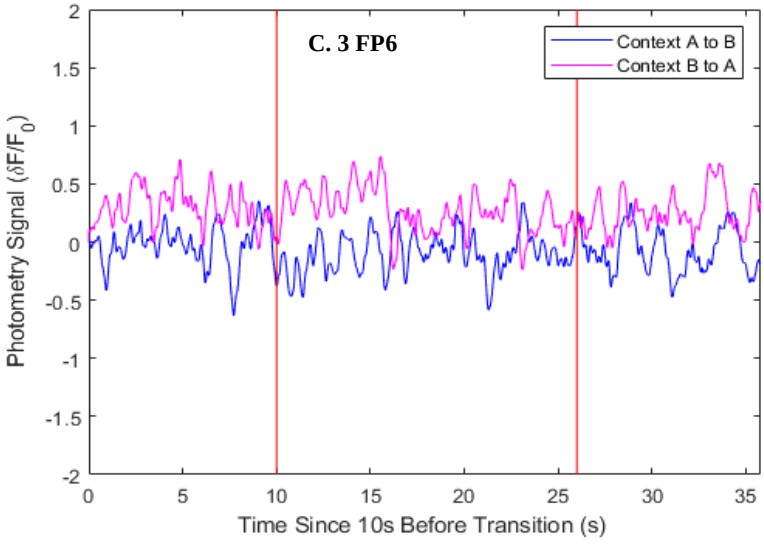
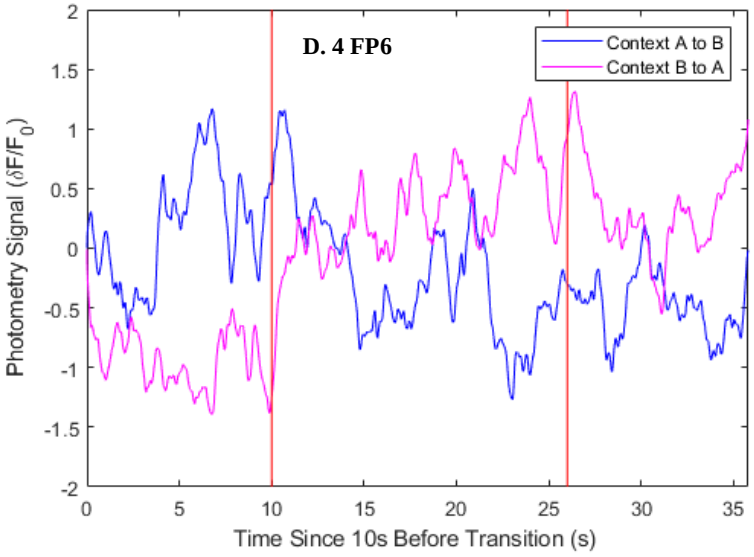


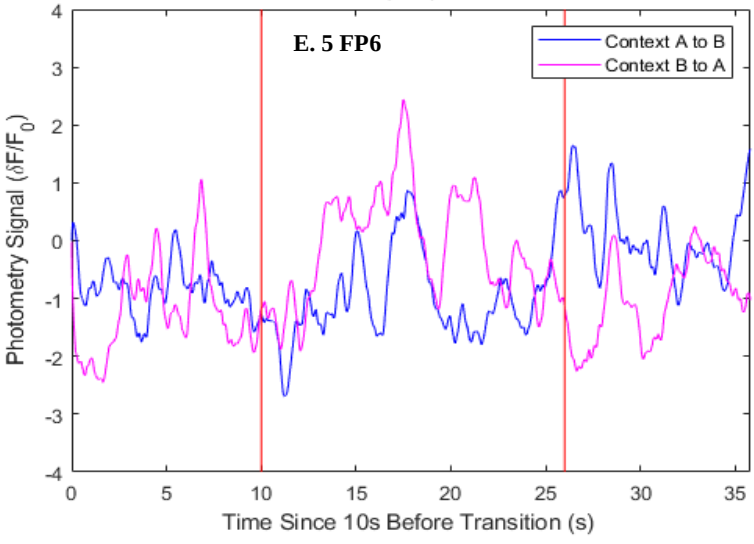
Figure 2: Behavioral Discrimination of Contexts. B_i and A_i corresponds to the i-th appearance of either context A or B during a run. Context B represents the conditioned context. Error bars represent +/- S.E.M across all mice during that epoch. * indicates significance with $p < 0.05$ for figure 1.D. Mice generally discriminated between the contexts and froze significantly more in context B than A.

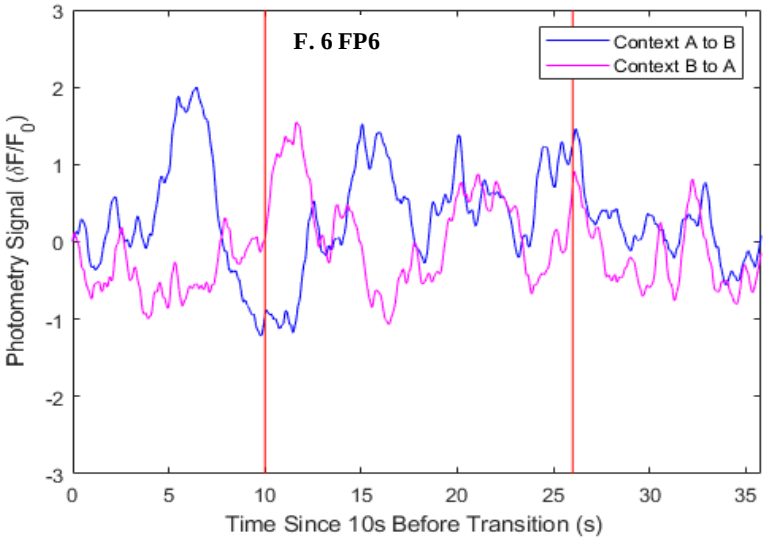

A. 1 FP6
Context A to B
Context B to A
Photometry Signal (δF/F0)
Time Since 10s Before Transition (s)

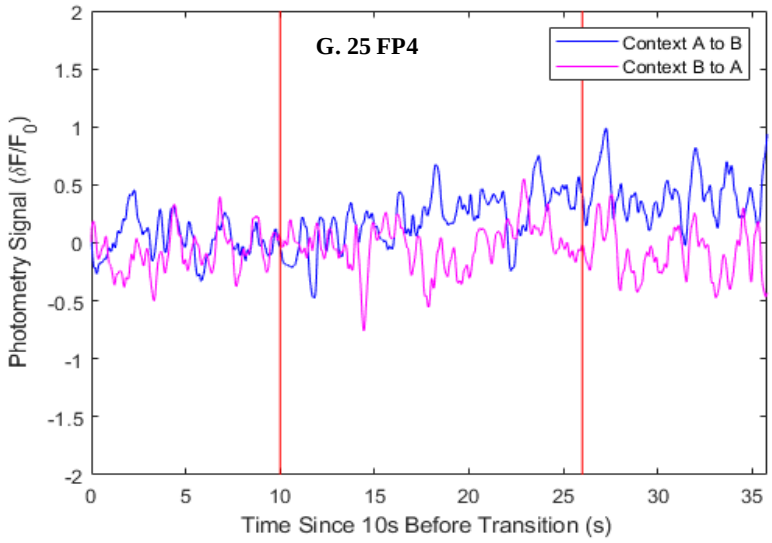

B. 2 FP6
Context A to B
Context B to A
Photometry Signal (δF/F0)
Time Since 10s Before Transition (s)

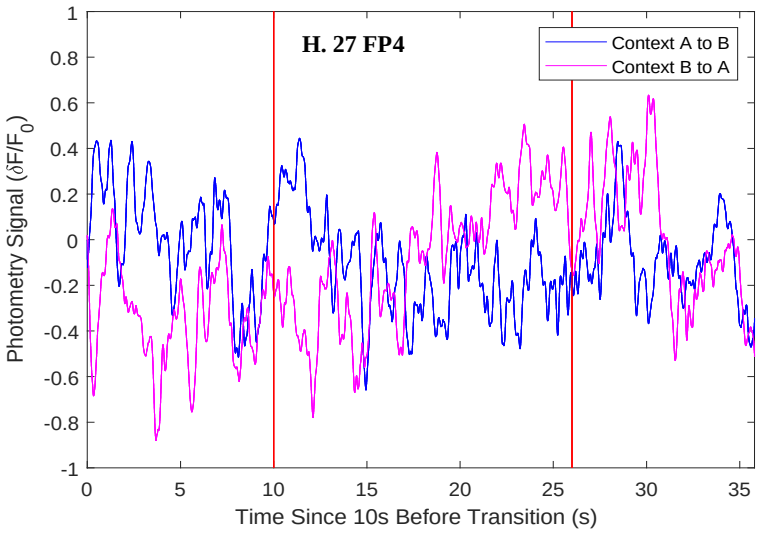

C. 3 FP6
Context A to B
Context B to A
Photometry Signal (δF/F0)
Time Since 10s Before Transition (s)


D. 4 FP6
Context A to B
Context B to A
Photometry Signal (δF/F0)
Time Since 10s Before Transition (s)


E. 5 FP6
Context A to B
Context B to A
Photometry Signal (δF/F0)
Time Since 10s Before Transition (s)


F. 6 FP6
Context A to B
Context B to A
Photometry Signal (δF/F0)
Time Since 10s Before Transition (s)





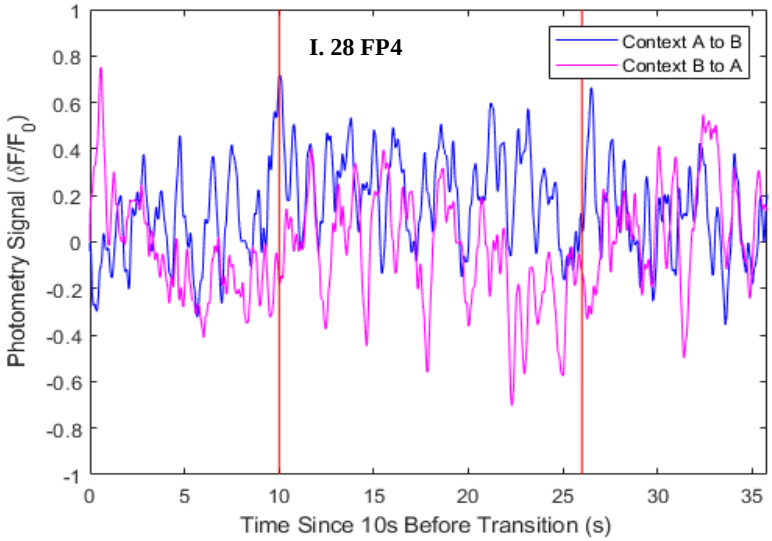


Figure 3: vCA1 activity during transition periods. Pink traces represent BA transitions and blue traces represent AB transitions. Each trace is the average signal over the 5 BA/AB transitions for a particular mouse. Red vertical lines represent the start and end of the 16 second transition period. The average signal 10 seconds before and after the transition period are given as context. These figures serve as a qualitative analysis of the vCA1 recordings during transitions.

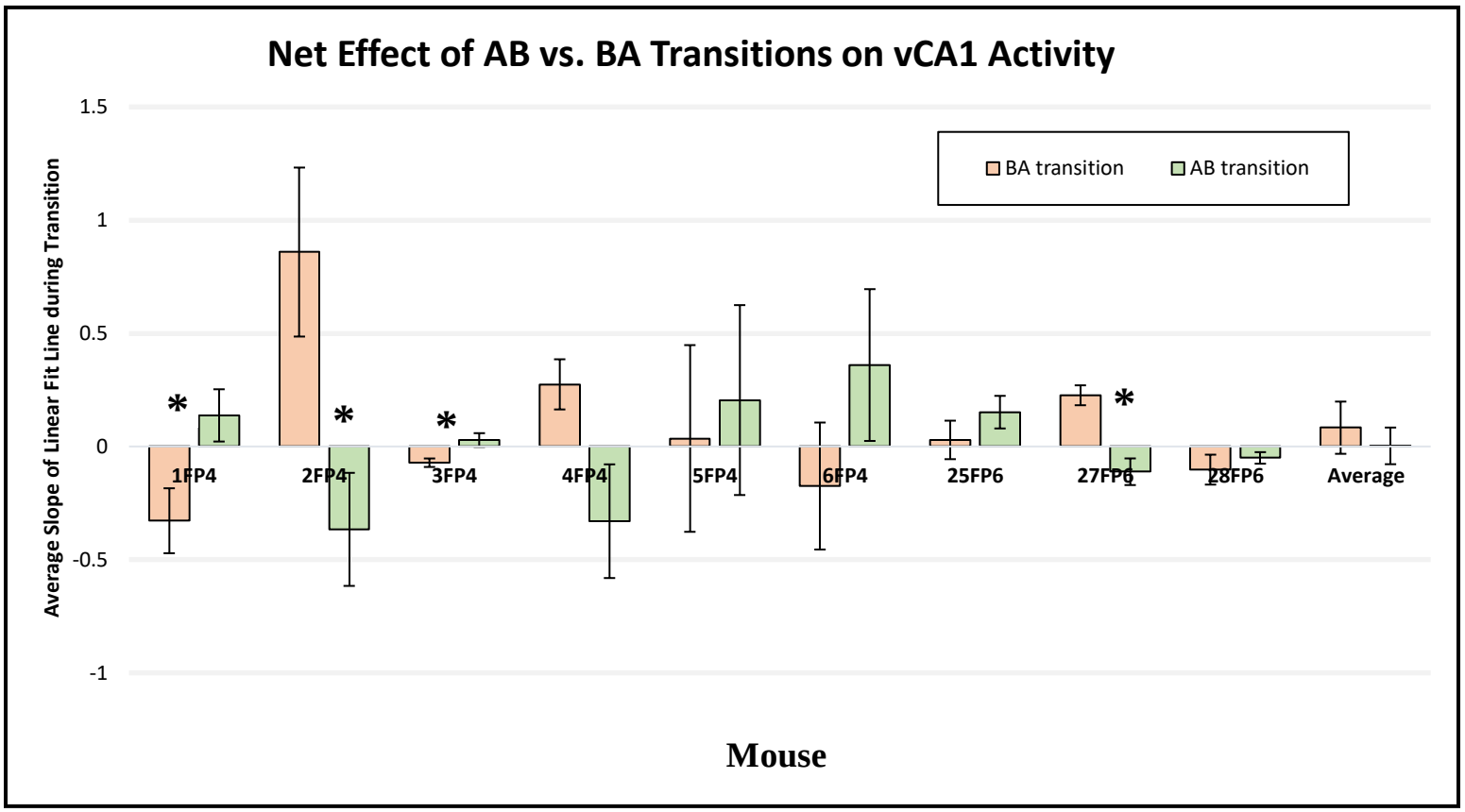


Figure 4: Net effect of different transition directions on vCA1 activity. During each transition, vCA1 activity was linearly fitted to test whether the transition induced more or less activation of surrounding neurons. The slope of this fitted line was observed for every transition. BA (beige) and AB (green) transition slopes were separated for each mouse and compared. Error bars show the +/- S.E.M. * indicates significance with $p < 0.05$. For 4 of the 9 mice, the direction of transition induced a significant change in slope. Transition direction did not induce a significant overall effect on vCA1 activity across mice.

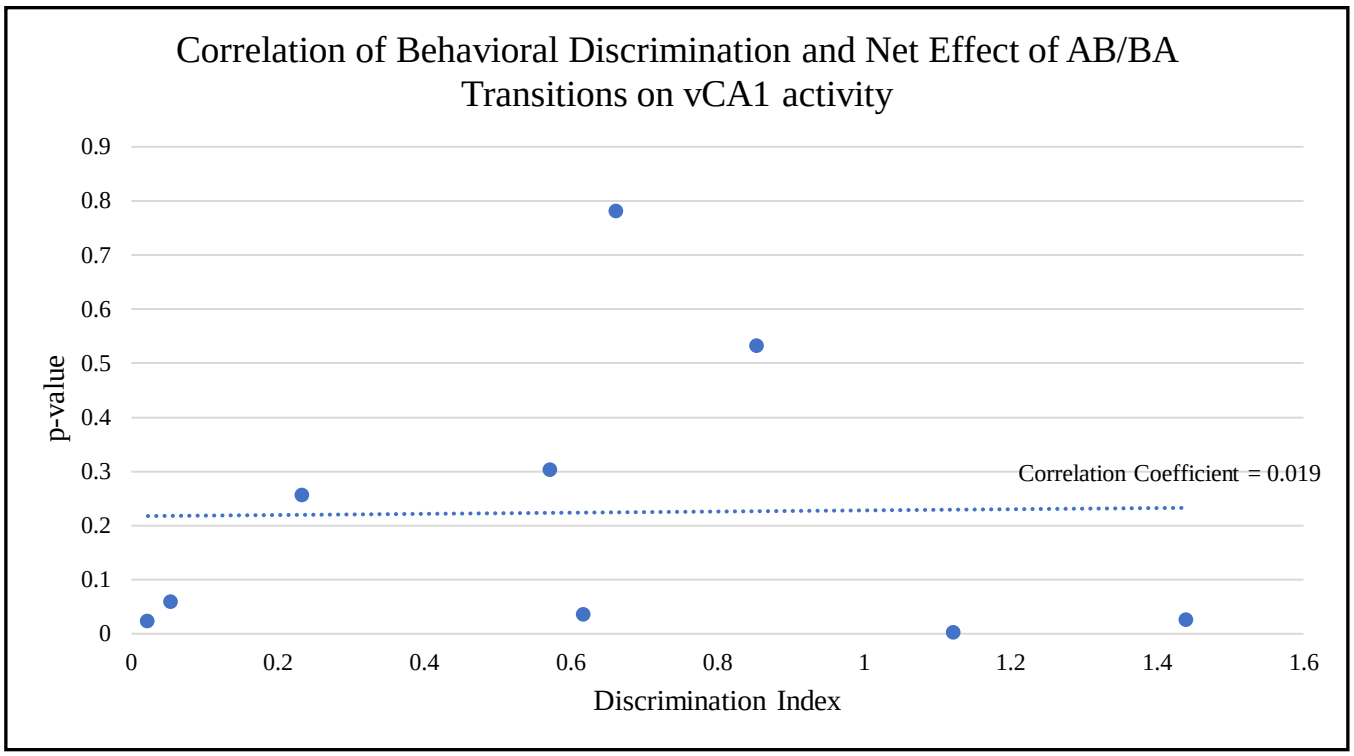


Figure 5: Correlation between p-values from AB vs. BA slope ANOVA and Discrimination Index. One-way ANOVAs were performed comparing AB vs. BA transition slopes. The p-values derived from these ANOVAs were correlated to each mouse's discrimination index (DI) (Table 1). The correlation between DI and p-values was not significant ($|\rho| < 0.70$). A mouse's overall behavioral discrimination did not predict its overall neuronal discrimination.

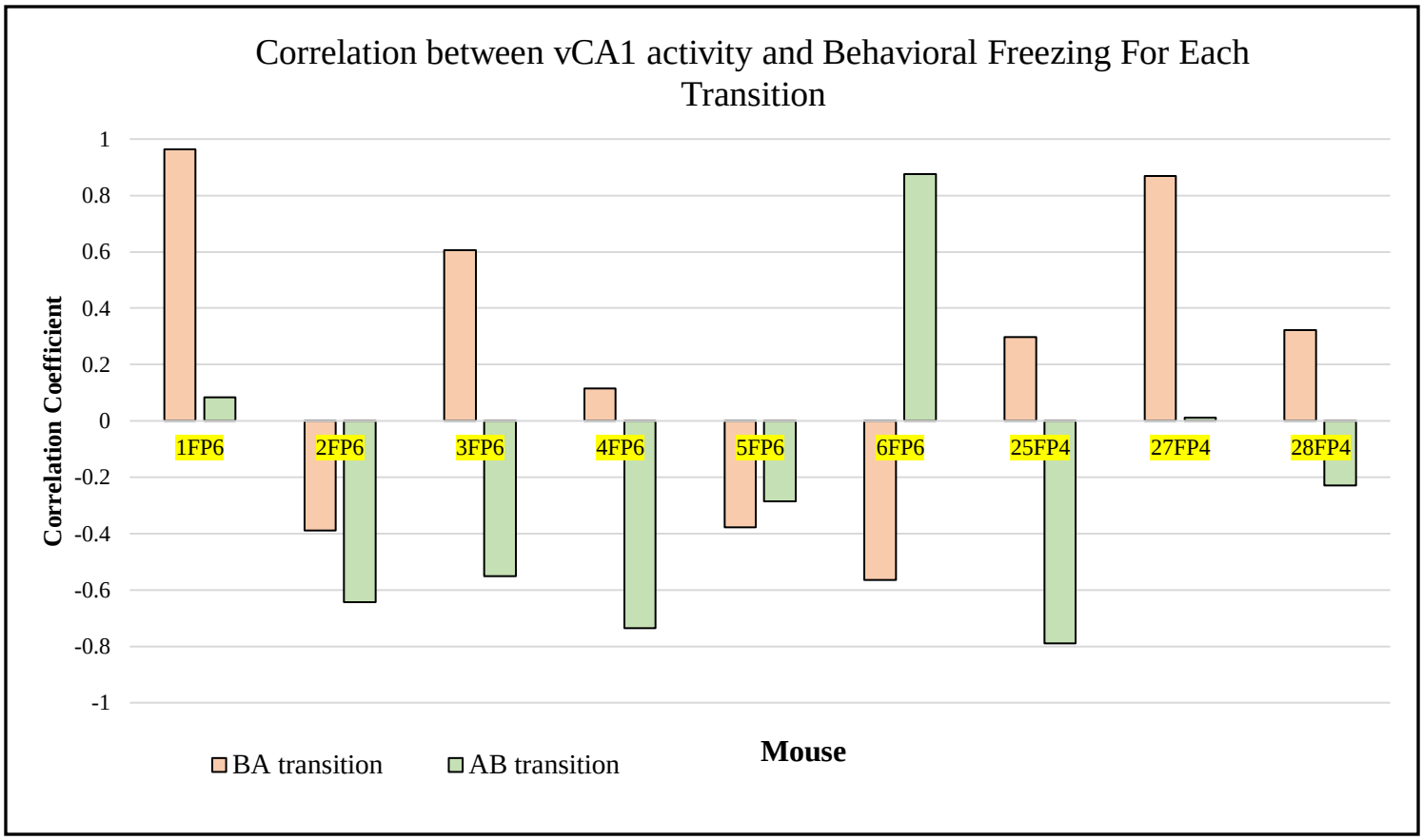


Figure 6: Correlation between vCA1 activity during a transition and behavioral freezing after that transition. Every transition period was sandwiched in between a 110s epoch in context A or B and a 110s epoch in the opposite context. For every transition, the slope of vCA1 change was recorded (Figure 4). The percent freezing in the context after the transition was subtracted from the percent freezing in the context before the transition. Correlation coefficients were observed between the differences in freezing after a transition and the slope of vCA1 activity during the transition. 5 of the 9 mice showed a significant correlation ($|\rho| > 0.70$) in either the AB direction or BA direction. No mice had significant correlations in both directions.

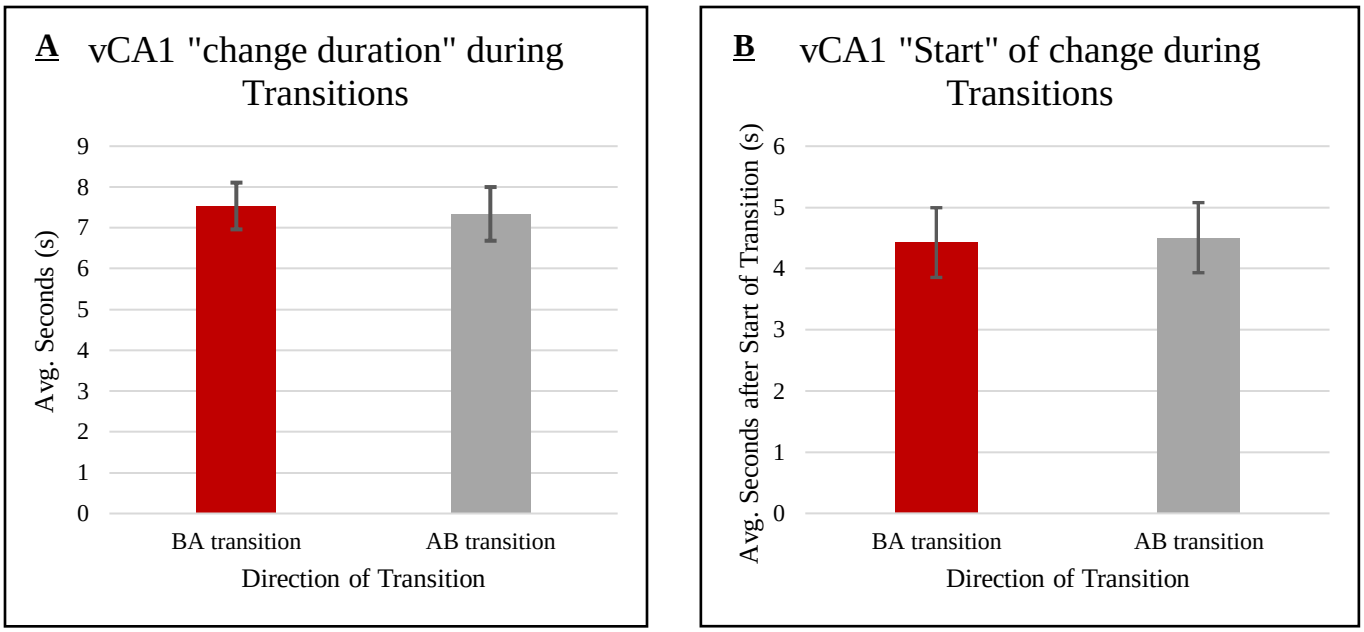


Figure 7: **Time component of vCA1 change due to transition.** During the 16 second transition periods, there is a 1 second window with the highest average calcium activity and a 1 second window with the lowest average calcium activity. The time between the middle of these 1 second periods is considered the "change duration" of vCA1 activity (figure 7.A) and the middle of the first 1 second period is considered the "start" of said change (figure 7.B). There were no significant differences for either measure between AB and BA transitions. Error bars represent +/- S.E.M.

Tables

Mouse	DI
1FP6	0.617057
2FP6	1.440056
3FP6	0.022616
4FP6	0.054546
5FP6	0.662243
6FP6	0.233581
25FP4	0.571977
27FP4	1.122362
28FP4	0.854381
Mean	0.619869

Table 1: **Discrimination Index**. A higher discrimination index (DI) represents a better behavioral discrimination (measured in freezing) between contexts A and B. A positive DI signifies more freezing in context B (the context that was coupled with foot shocks). So, 2FP6 discriminated the best and 3FP6 discriminated the worst between contexts.

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