이학박사학위논문

편도체의 감정 가치 정보 생성기전

Valence encoding in the basal amygdala

2019년 8월

서울대학교 대학원

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Abstract

Valence encoding in the basal amygdala

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The amygdala is an essential part of the brain region, which processes emotional information. Among different forms of emotion, innate and learned fear have been extensively studied using in vitro and in vivo electrophysiology. Moreover, the amygdala is also involved in other emotional memory processes such as reward memory, anxiety, and social interactions.

During the emotional memory process, the amygdala is known to encode valence and arousal of emotional stimuli, which represent emotional value and attention of the stimuli, respectively. More specifically, different valences are encoded by a distinct neuronal population of the amygdala that project to the different brain area, respectively, while arousal is encoded by the same neuronal population of the amygdala irrespective of valences of the emotional stimuli.

However, in most of the previous studies, researchers have recorded amygdala neuronal activities only at a particular time point, and hence it has been challenging to test the possibility that the amygdala involves a time-dependent process such as encoding of events.

In my thesis, I adopted a long-term recording of the single neurons in the basal amygdala to test the possibility.

In chapter 1, I confirmed that reward and fear conditioning recruited different subpopulations in the basal amygdala, consistent with previous studies. I further tested whether the extinction of reward and fear conditioning also involved different subpopulations, respectively. Extinction of reward and fear conditioning appeared to involve different subpopulations which are segregated from those recruited during reward and fear conditioning. These findings suggest the possibility that extinction involves changes in valence but not in

arousal since recruitment of different subpopulations underlies extinction of reward and fear conditioning.

However, it is also possible that the basal amygdala encodes events instead of values because the subpopulations show neural activity alteration as a session-specific manner.

Accordingly, in chapter 2, I determined whether the basal amygdala encodes events. For this, I stably recorded single neurons in the basal amygdala while I repeated two sessions of fear conditioning. I found that the repeated conditioning recruited different subpopulations in the basal amygdala. Furthermore, I tested whether repeated retrieval after a single session of fear conditioning recruited different subpopulations in the basal amygdala. As a result, I found that repeated retrieval also recruited different subpopulations. Together, my findings suggest event-specific encoding in the basal amygdala.

Key words: Basal amygdala, value, valence, arousal, fear conditioning, reward conditioning

Student Number: 2013-23004

Contents

Page

List of figures

Page

Chapter 1.

Chapter 2.

Background and Hypothesis

1. Background

1.1. Emotional learning and amygdala

Animals experience various emotional events, so it is crucial to memorize those emotional events correctly. For example, an appropriate response to the fear stimuli helps animals to survive, whereas overexpression of the fear response interferes in ordinary life. The amygdala is the most intensely studied brain area related to the emotional memory process. The amygdala locates on the temporal lobe of the brain and is composed of several different regions such as lateral (LA), basal (BA), accessory basal (AB), and central nuclei (LeDoux, 2000). The basolateral amygdala (BLA), which is composed of lateral (LA) and basal (BA) part of the amygdala, is a well-known place for associative learning. The importance of the BLA related to associative learning is revealed by lesion and electrophysiological studies (Hatfield et al., 1996; Phelps and LeDoux, 2005; Quirk et al., 1995; Tye et al., 2008). Another subdivision of the amygdala is central amygdala (CeA) where fear-related behavior output pathway (Ciocchi et al., 2010; Wolff et al., 2014).

A simplified associative learning process related to the amygdala is as follow. Environmental stimuli, usually indicate neutral conditioned stimuli (CS) and aversive or appetitive unconditioned stimuli (US), convey to the LA by sensory thalamus and sensory cortex (Quirk et al., 1997). When CS and US input arrives at the

same synapse within the LA, CS-US association occur. Then, the LA sends the emotional information to the other amygdala sub-regions, BA and CeA (Maren and Fanselow, 1996). The BA has lots of reciprocal connection with multiple brain regions, especially cortical areas such as medial prefrontal cortex (mPFC) and orbitofrontal cortex (OFC) (McDonald, 1998). By doing so, the BA modulates emotional information. After those emotional information process, BLA sends the information to the downstream brain regions such as CeA and nucleus accumbens (NAc), which induce behavior output followed by environmental stimuli (Hu, 2016) (Fig. 1).

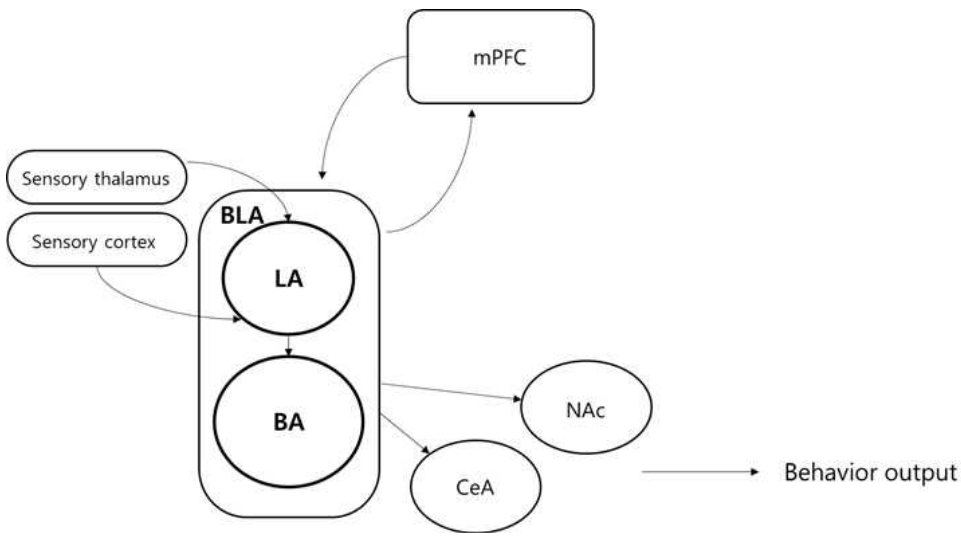


Figure 1. A simplified representation of the neural pathway related to BLA.

The basolateral amygdala (BLA) has a massive connection with other brain areas. Lateral amygdala (LA) gets an environmental input from sensory thalamus and cortex. Within BLA, LA conveys information to the basal amygdala (BA). BA has a reciprocal connection with the cortical areas, especially medial prefrontal cortex (mPFC). BLA sends the neural signal to downstream brain regions such as the central amygdala (CeA) and nucleus accumbes (NAc) to induce behavior output.

1.2. Fear conditioning and extinction

1.2.1. Fear conditioning

Fear memory is vigorously studied emotional memory by using fear conditioning because fear conditioning causes a srapid and straightforward response to animals (Fig. 2A). During auditory fear conditioning, the association between neutral conditioned stimuli (CS) and aversive unconditioned stimuli (US) occur within the LA (Quirk et al., 1997). Before fear conditioning, CS does not induce a strong response to the LA. However, the synapse is strengthened after fear conditioning by CS-US association. Therefore, CS causes a rapid and robust response to the LA. In vitro electrophysiology indicated that synapse from sensory thalamus and sensory cortex showed long term potentiation (LTP) (Blair et al., 2001; McKernan and Shinnick-Gallagher, 1997) while in vivo electrophysiology data showed that rapid and robust response appeared as a result of fear conditioning (Quirk et al., 1995; Repa et al., 2001). Moreover, lesion studies indicated that fear behavior disappeared by inactivation of the LA (Phelps and LeDoux, 2005; Phillips and LeDoux, 1992). Recently, by using the optogenetic technique, artificial reinforcement of the synapse within the LA made false fear memory and induced defensive behavior (Johansen et al., 2014; Johansen et al., 2010).

CeA is another sub-region of the amygdala which locates on the downstream of the BLA. The CeA acquires information from the

BLA and causes defensive behavior. Electrophysiological and lesion studies support the role of the CeA, which induces fear response (Ciocchi et al., 2010; Maren and Quirk, 2004; Nader et al., 2001). Moreover, recent studies showed that stimulating the pathway from the BLA to the CeA induced fear response (Namburi et al., 2015).

Other brain areas, such as prelimbic cortex (PL), are also crucial for the fear conditioning process. Inactivation of the PL disrupted fear conditioning process. At the same time, robust neural response followed by CS presentation appeared as a result of cue-dependent fear conditioning (Corcoran and Quirk, 2007). Overall, lots of brain regions are involved in the fear conditioning process. Among them, the amygdala and the PL, which interact with each other are the most well-known brain regions related to the fear conditioning process (Burgos-Robles et al., 2009; Herry and Johansen, 2014; Marek et al., 2013; Sotres-Bayon and Quirk, 2010; Tovote et al., 2015).

1.2.2. Fear extinction

Fear extinction is behavior paradigm, which alleviates fear response by exposing to the CS without the US after fear conditioning (Fig. 2B). BLA is an essential part of the fear extinction process. Previous studies demonstrated that synapse, which was strengthened after fear conditioning, was weakened followed by fear

extinction (Hong et al., 2009; Kim et al., 2007). In vivo electrophysiology also showed that neurons, which exhibited an excited response after fear conditioning, displayed diminished response after fear extinction. At the same time, the reinforcement of the neural response also occurred within the BLA as a result of fear extinction (Herry et al., 2008). Furthermore, lesion studies indicated that inactivation of the BLA blocked fear extinction process and animals maintained fear response (Sierra-Mercado et al., 2011).

During the fear extinction process, the BLA interacts with other brain areas such as the infralimbic cortex (IL) and the ventral hippocampus (vHC). IL locates on the medial prefrontal cortex (mPFC) and has a reciprocal connection with BLA (McDonald, 1998). Previous data indicated that inactivation of IL and vHC impaired fear extinction process (Sierra-Mercado et al., 2011) while neural activity alteration appeared within the IL followed by fear extinction process (Milad and Quirk, 2002).

Together, the amygdala is an essential place for encoding fear memory, which includes both fear conditioning and extinction. Neural response keeps changing after fear conditioning and extinction, while inactivation of the amygdala blocks the behavior alteration. Also, other brain regions, especially mPFC and vHC affect to the emotional memory process by interacting with the amygdala.

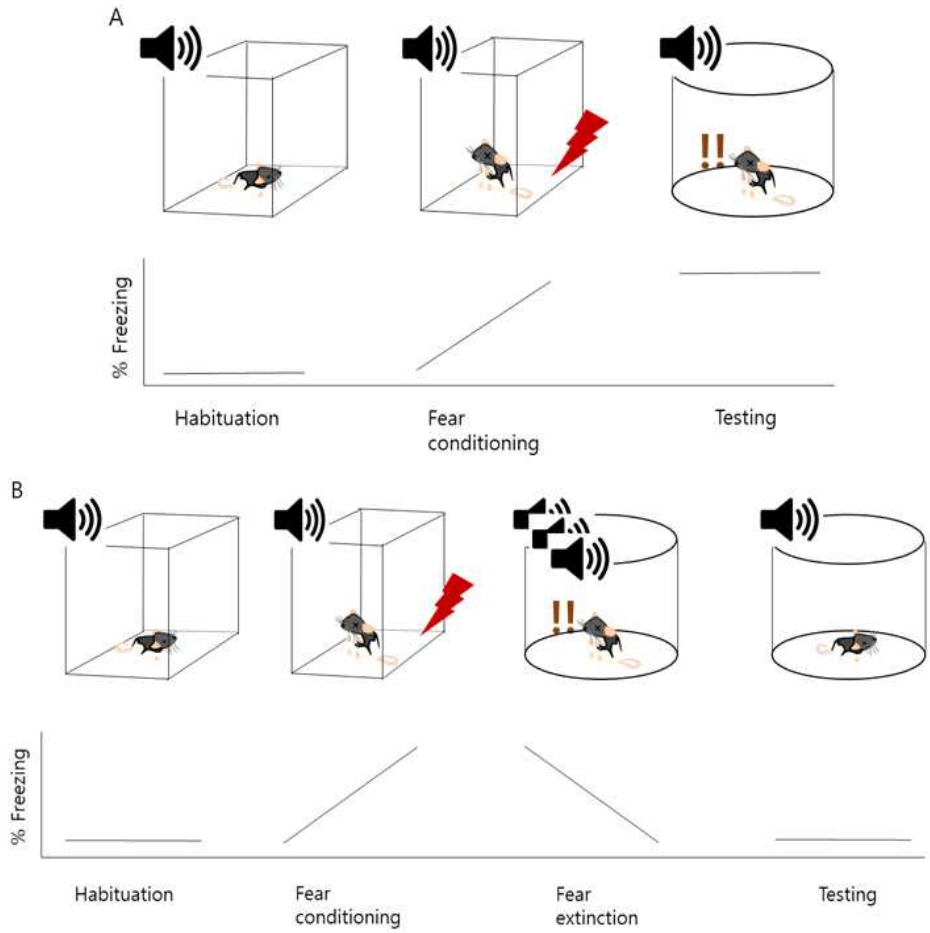

A
% Freezing
Habituation
Fear conditioning
Testing
B
% Freezing
Habituation
Fear conditioning
Fear extinction
Testing

Figure 2. Pavlovian fear conditioning and extinction.

A. Pavlovian fear conditioning paradigm in rodents. Before fear conditioning, rats do not show freezing response to neutral tone CS. During fear conditioning, rats associate CS and foot-shock US and exhibit freezing response to the CS at the end of fear conditioning. Freezing response to the CS is maintained until the testing. **B.** Pavlovian fear extinction paradigm in rodents. After fear conditioning, rats are exposed to multiple CS without foot-shock. Freezing response to the CS diminish during extinction session.

1.3. Reward conditioning and extinction

1.3.1. Reward conditioning

The amygdala is involved in the emotional process generally. Therefore, the amygdala also plays a crucial role in the reward memory process. However, the majority of the previous studies related to the amygdala focus on the aversive emotional memory process because of Pavlovian fear conditioning paradigm, which is a simple and effective behavior protocol for the experiment. Recently, some of the evidence indicates that reward-related emotional process is also related to the amygdala (Belova et al., 2007; Paton et al., 2006; Tye et al., 2010; Tye et al., 2008). Pavlovian reward conditioning is one of the reward conditioning process (Fig. 3A) but instrumental conditioning, which induces instrumental response as a result of learning, also use commonly.

Same as fear conditioning, BLA is a crucial place associating a positive value with neutral CS. Therefore, inactivation of the amygdala interrupted reward conditioning process (Hatfield et al., 1996; Hiroi and White, 1991). Moreover, in vitro electrophysiology studies indicated that synapse within the LA was strengthened followed by reward conditioning while in vivo electrophysiological data showed a rapid and robust response to the CS after reward conditioning (Tye et al., 2008).

Other brain structures such as mPFC and orbitofrontal cortex

(OFC) also affect the reward memory process by interacting with the amygdala (Berridge and Robinson, 2003; Holland and Gallagher, 2004; Salzman and Fusi, 2010; Schultz, 2000). Lesion studies indicated that loss of mPFC or OFC caused inappropriate response during an emotional memory task (Bechara et al., 1994; Stalnaker et al., 2007). Furthermore, electrophysiology studies suggested that the neural activity of the mPFC and OFC had a close relationship with reward memory process (Schoenbaum et al., 1998).

Nucleus accumbes (NAc), which is a part of the striatum, also involves in the reward memory process by sending behavior output signals (Hu, 2016). Furthermore, recent studies demonstrated that stimulating neural pathway from the BLA to the NAc induced reward-related behaviors (Namburi et al., 2015; Stuber et al., 2011).

1.3.2. Reward extinction

Same as fear extinction, reward extinction also exposes animals to the multiple CS presentation without reward outcome (Fig. 3B). By doing so, animals lose their interest in the CS and reward port approach, which increased after reward conditioning.

Neural response alteration also appeared within the BLA followed by reward extinction. In vitro studies suggested that strengthened synapse was weakened as a result of reward extinction (Rich et al., 2019). At the same time, in vivo electrophysiology

studies showed that part of the BLA neurons activated as a result of reward extinction (Sangha, 2015; Tye et al., 2010). Lesion studies also indicated that the BLA inactivation blocked CS-dependent value alteration process (Hatfield et al., 1996). Moreover, the cortex area, especially OFC, has an essential role in the devaluation process. Lesion of the OFC disrupted a behavior change followed by value alteration (Pickens et al., 2003).

Overall, fear and reward emotional process mediate similar synapse response within the amygdala. Therefore, the role of the amygdala during emotional memory process is not narrow down to the aversive memory process. By using similar neural process, the amygdala helps animals to associate the environmental stimuli with a specific value and predict the outcome followed by the stimuli.

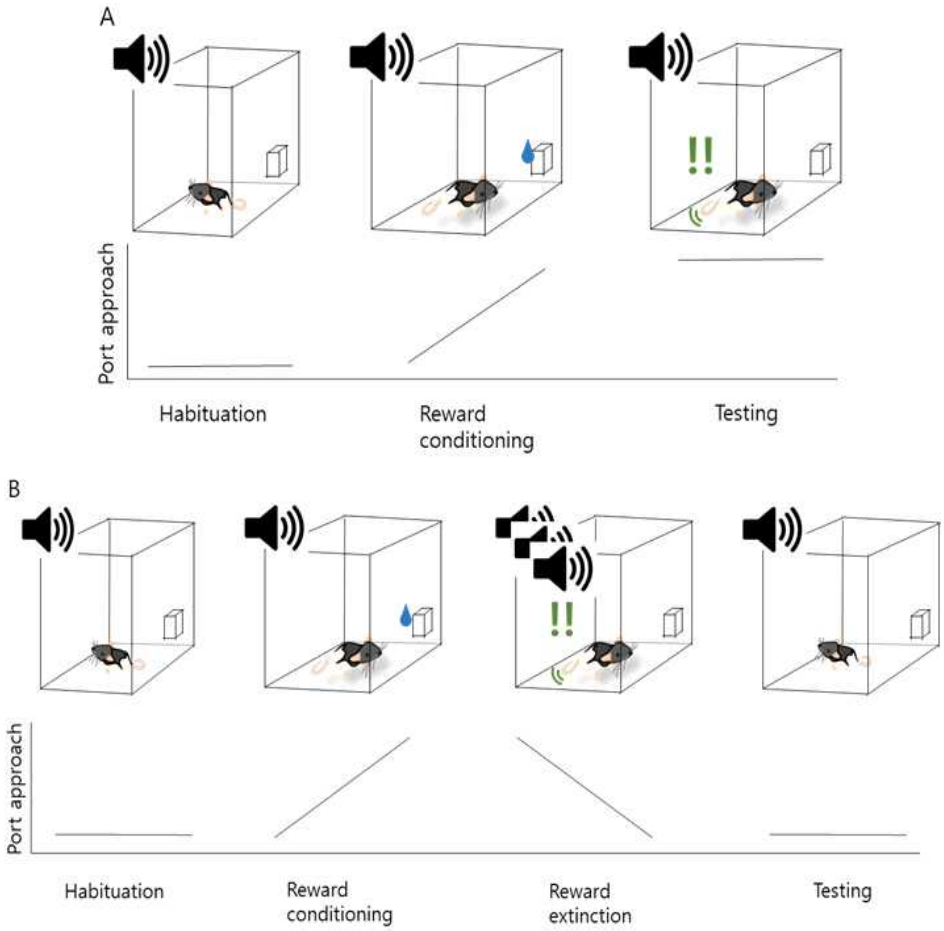

A
Port approach
Habituation
Reward conditioning
Testing
B
Port approach
Habituation
Reward conditioning
Reward extinction
Testing

Figure 3. Pavlovian reward conditioning and extinction.

A. Pavlovian reward conditioning paradigm in rodents. Before reward conditioning, rats do not interest in reward port during neutral tone CS presentation. After rats associate CS and reward US, they display the reward port approach during CS presentation. Reward port approach behavior is maintained after reward conditioning. **B.** Pavlovian reward extinction paradigm in rodents. After reward conditioning, rats are exposed to multiple CS alone. Reward port approach to the CS disappears after extinction sessions.

1.4. Valence and salience

Among the model of emotional coding, one indicates that valence and salience compose emotion. Followed by this model, valence represents a positive or negative value, while salience represents the intensity of the stimuli. Combination of the valence and salience compose different emotion and Fig. 4 show some of the examples of the emotion (Russell, 1980).

To figure out the existence of valence and salience components within the amygdala, experiments conducted by using two different stimuli, which associated with a positive and negative outcome, respectively. By recording the neural activity during associative learning with two different stimuli with opposite value, the existence of the value coding neurons within BLA was revealed (Belova et al., 2007; Gore et al., 2015; Paton et al., 2006). From these experimental results, neurons, which respond to the specific stimuli, is regarded as representing the valence of the emotion. Recent studies defined valence neuron more precisely. Experimental data suggested that positive valence neurons show excited neural response to the positive cue while inhibited or no neural response appeared to the negative stimuli. The opposite neural response appeared for negative valence neurons, which showed excited response to the negative stimuli while inhibited or no neural response appeared to the positive stimuli (Beyeler et al., 2016). Together, experimental results suggest that valence information is encoded by distinct neural population.

Representation of the intensity of the stimuli is called salience. In contrast to the valence, salience affects the autonomic system activity and alter the neural activity to both positive and negative cue (Gallagher and Holland, 1994; Sengupta et al., 2018; Shabel and Janak, 2009). The mechanism of the valence and salience coding is uncertain because different valence and salience are encoded as intermingled neurons within the amygdala (Beyeler et al., 2018; Kim et al., 2016; Shabel and Janak, 2009). However, the evidence still suggests that neurons, which represent valence and salience, exist within the amygdala and combination of this neural activity contribute to encoding emotion.

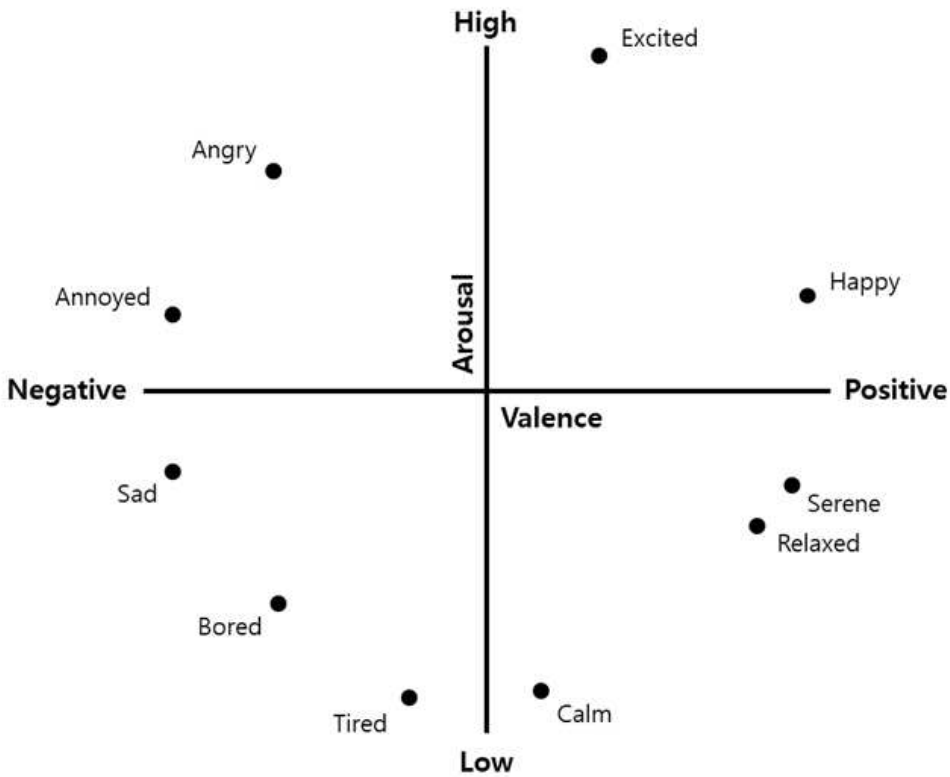


Figure 4. Two dimension scaling of arousal versus valence.

One of the common models of emotion. Valence represents the value of the emotion and arousal represent attention. Emotion is composed of combination with valence and arousal. Few example words are plotted on the two dimensions.

2. Hypothesis

The amygdala is an essential part of the brain region, which animals encode emotional memory. Within the amygdala, the distinct neural subpopulations appear as a result of emotional learning (Herry et al., 2008; Tye et al., 2008). At the same time, the independent neural population also appear when the valence of the emotion is encoded (Beyeler et al., 2016). Furthermore, recent studies indicated that a distinct neural population within the amygdala with different connection induced valence-dependent behavior output (Gore et al., 2015; Namburi et al., 2015).

However, the role of the amygdala during value coding process is uncertain because previous studies have focused on the neural response alteration at specific-session.

Moreover, lots of previous studies examine the basolateral amygdala (BLA) even though anatomical and physiological properties are different between lateral (LA) and basal (BA) amygdala (McDonald, 1998). Especially, LA neurons tend to maintain a stable response during the emotional memory process (An et al., 2012; Han et al., 2009; Reijmers et al., 2007).

Based on the previous data, I hypothesize that the BA encodes emotional event via distinct neuronal subpopulations. To test the hypothesis, I observed neural activity modification by recording

single unit activity during the entire behavior sessions for three different behavior paradigm: CS-dependent value updating, repeated fear conditioning and consecutive two sessions of retrieval after a single fear conditioning (Fig. 5).

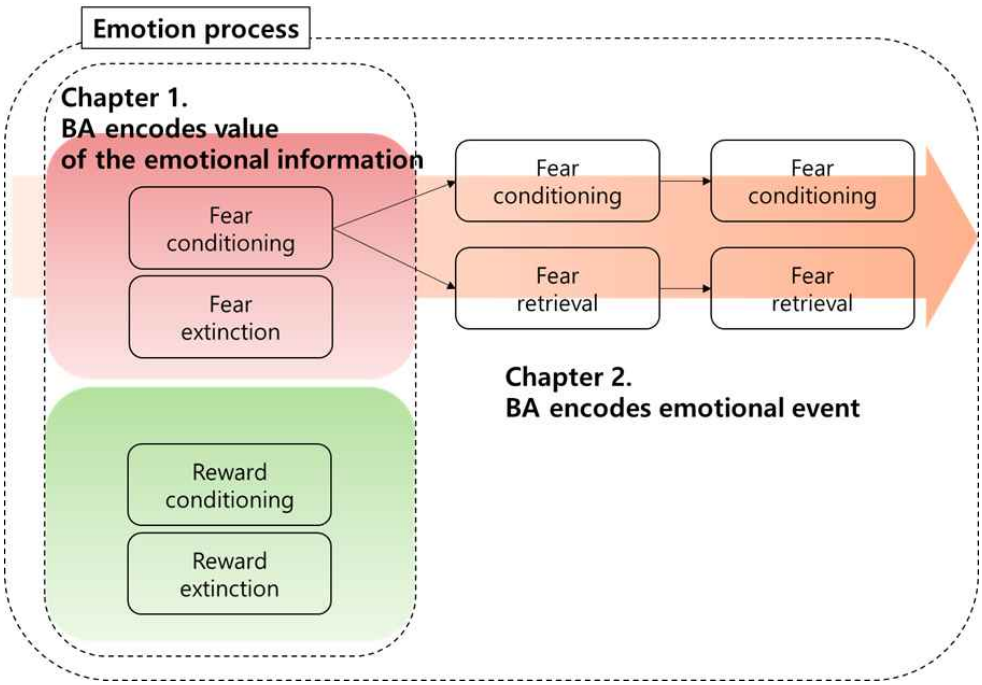


Figure 5. Schematic diagram of the research.

In chapter 1, I examined whether BA encodes the value of the emotion by observing neural activity alteration during value changes by fear and reward behavior process. In chapter 2, I tested whether BA encodes emotional events by tracking down the neural activity during repeated fear conditioning or two consecutive retrievals.

Chapter 1.

Basal amygdala (BA) encodes emotional information by updating current value via distinct neural populations

Abstract

Basal amygdala (BA) is the brain area which processes appetitive and aversive emotional memory. Neurons, which represent appetitive and aversive emotional memory, appear in the BA but little is known how neural activity change occurs during cue-dependent reward and fear learning and alteration of the value of the cue. Here I demonstrate that a large proportion of learning-dependent CS-onset responsive neurons respond reward-related or fear-related cue, which is known as valence neurons, followed by emotional behavior training (about 75%). Moreover, during the entire appetitive and aversive behavior training, I found out that most of the valence neurons show a session-specific response, which response after the conditioning or extinction specifically. These data suggest that BA neurons encode current emotional value via distinct neural population.

Key words: Valence, arousal, basal amygdala, value updating

Introduction

Animals frequently undergo a positive and negative emotional experience. Therefore, it is essential to memorize those experiences and apply to a similar situation to get the best outcome. By doing so, animals need to encode the value of emotional experience.

Amygdala is a well-known place for the appetitive and aversive emotional memory process (Janak and Tye, 2015; LeDoux, 2000; Phelps and LeDoux, 2005). Ex vivo electrophysiology and in vivo single unit data indicate that neurons within the amygdala exhibit learning dependent neural plasticity change to the aversive associative learning (Blair et al., 2001; McKernan and Shinnick-Gallagher, 1997; Quirk et al., 1997; Quirk et al., 1995; Rogan et al., 1997). At the same time, previous studies indicate that appetitive behavior training also alters neural activity within the amygdala (Baxter and Murray, 2002; Murray, 2007; Tye et al., 2010; Tye et al., 2008).

After cue-dependent emotional learning, amygdala neurons alter conditioned stimuli (CS)-onset response as a result of the CS and unconditioned stimuli (US) association. Those neurons show either the emergence of CS-onset response or alteration of neural activity after emotional learning compared to the baseline (Goosens et al., 2003; Grundemann and Luthi, 2015; Herry et al., 2008; Repa et al.,

2001).

Neurons, which alter CS-onset response after appetitive and aversive learning, is composed two different components: both (same) cue response neurons, which show CS-onset response to both appetitive and aversive cue, and cue-specific response neurons, which display CS-onset response either appetitive or aversive cue. Previous studies reveal that neurons which exhibit CS-onset response to both appetitive and aversive cue after reward and fear conditioning represent arousal, which represents the attention of the stimuli (Shabel and Janak, 2009). On the other hand, neurons, which display a specific response to the appetitive or aversive cue, represent valence information, which represent value of the stimuli (Balleine and Killcross, 2006; Belova et al., 2007; Beyeler et al., 2016; Namburi et al., 2015; O'Neill et al., 2018; Redondo et al., 2014). These findings indicate that separate neural populations exist within the amygdala, which is divided by their function of the value coding process.

Majority of previous studies related to neural response alteration after emotional learning focus on either appetitive or aversive behavior. By doing so, the existence of conditioning and extinction neurons for appetitive or aversive CS was found (Herry et al., 2008; Tye et al., 2010; Tye et al., 2008). However, animals usually confront a complicated emotional situation which is comprised of mixed stimuli with different emotional value. Moreover, those cue specific emotional values keep changing. Some of the previous studies

show neural response when animals perform both appetitive and aversive behavior, and cue-dependent value is altered by extinction (Livneh and Paz, 2012; Sangha, 2015), but there is no direct evidence for the role of neural response alteration when animals experience opposite cue-dependent learning, and cue-related value is varied. Therefore, to understand the role of the amygdala when the animals confront the complicated emotional situation, it is necessary to survey neural activity modification depend on the emotional value change.

In this study, I examine how basal amygdala (BA) neural plasticity is altered when appetitive and aversive cue obtains specific value by emotional learning and the cue-dependent value changes. By doing so, I conducted cue-specific reward and fear conditioning to make CS-dependent emotional information. After the consolidation of reward and fear memory, I carried out reward and fear extinction for changing CS-dependent value, which was created by conditioning. By tracking down the neural activity during the entire behavior session, I observe the neural activity alteration while the cue-dependent value is varied.

Materials and Methods

Animals. Male Sprague Dawley rats (n=43, 8 weeks old) were individually housed for 5-7d before experiments under an inverted 12h light/dark cycle (light off at 9:00 a.m.). Food and water were restricted for 4d prior to the experiment. Animals were handled twice a day for 3 days. During the last 2 days, the reward port was used to improve port access. Behavior training was conducted during the dark proportion of the light/dark cycle. Entire procedures were approved by the Institute of Laboratory Animal Resources of Seoul National University.

Behavior apparatus and procedures. Reward conditioning and extinction were conducted in a rectangular context with the different floor (context A; grid, context A'; flat, Fig. 6A). Fear conditioning and extinction were conducted in circular context with the different floor (context B; grid, context B'; flat, Fig. 6A). Reward and fear conditioning was done in a different context to minimize the contextual association between reward and fear behavior task. Context A and A' were a rectangular opaque box (23.5㎝ lengths x 23㎝ widths x 27㎝ height) which was illuminated with white light and cleaned with 70% ethanol solution. Two reward ports located 2.5 ㎝ above the floor in the center of the side walls. Context B and B'

were Plexiglas cylindrical chamber (27㎝ lengths x 27㎝ widths x 30㎝ height) which was illuminated with red light and cleaned with water and sprayed 1% acetic acid. The LED light located 15cm above the floor. Metal grid connected to an electrical current source (Coulbourn Instruments) and context was situated in a sound attenuating chamber.

On day 1, rats were habituated context A' and B'. Animals were placed in each context for 20 min. Rats stayed without cue for the first 10 min, and five cues were presented after 10 min. 10s continuous 2.8 kHz tone (85dB) and 10s continuous light were used for reward and fear related task, respectively. On day 2, rats were given ten presentations of the tone and light CS in each context to determine basal amygdala neural responses to the CS (Habituation). 8 hours later, reward conditioning was conducted by pairing the CS with a 15% sucrose reward which was offered 5s after tone onset (5s duration; 50 CS/US parings; inter-trial interval; 65-85s). On day 3, rats were given second reward conditioning and 8 hours later fear conditioning was conducted by pairing the CS with an electric foot shock (0.5mA, 0.5s, 5 CS/US pairings, inter-trial interval; 65-85s) co-terminating with the offset of the light CS. On day 4, reward extinction was conducted with 30 non-reinforced CS presentation (post-RC) and 8 hours later fear extinction was conducted with 30 non-reinforced CS presentation (post-FC). On day 5, the behavioral and neuronal outcome of reward and fear extinction sessions were observed in a ten CS test session (post-RC EX and post-FC EX).

All of the training sessions were videotaped except two rats, which missed 3 CS response during pre-FC because of video program was stopped. Trained observers quantified nose-poking and conditioned freezing. The animals were regarded to be freezing when there was no movement except for respiratory activity for 1s during 10s CS presentation. The total freezing time normalized to the duration of the CS presentation (An et al., 2012; Kim et al., 2010). The animals were considered to access the reward port when their nose enter the port during 10s CS presentation. Percent trials in port normalized to the ten trials of CS presentation and represented to a block. Rats which didn't access to the port during the first five trials of CS presentation from reward conditioning result were removed from analysis to confirm the behavior results.

Surgery. Rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50mg/kg) and maintained with isoflurane (1-1.5%) in O_2 . Rats were mounted on a stereotaxic apparatus (Storting) and bilaterally implanted with fixed-wire electrodes targeted to the BA (anterior-posterior; -2.85mm, medial-lateral; ±5.10mm and dorsal-ventral; -8.85mm). The electrodes consisted of eight insulated nichrome microwires (50μm outer diameter, impedance 0.2-0.5 MΩ; California Fine Wire), which contained in a 21 gauge stainless steel guide cannula. The electrodes were affixed to the skull with dental cement (Vertex). After surgery, analgesic (Metacam;

Boehringer) and antibiotics (KOCHA CETIO) were injected. Rats were allowed to recover for 9-11d.

In vivo single unit recording. Neural response was acquired and analyzed using a Plexon MAP system (An et al., 2012; Herry et al., 2008). Unit discrimination was conducted by using Offline Sorter (Plexon). All waveforms were represented in a principal component space. The clusters with similar waveforms were first defined automatically and verified manually. A cluster of waveforms segregate from other clusters in principal component space was considered to be generated from a single neuron. Single channel recordings proved sufficient to discern single-unit responses, due to the low neuronal density of the amygdala (An et al., 2012; Pare et al., 2004; Quirk et al., 1997). The long-term stability of single-unit was determined by using Wavetracker (Plexon), which showed the principal component space cylinders maded by a unit recorded from different sessions (An et al., 2012; Herry et al., 2008; Tseng et al., 2011). A vertical cylinder suggests that the clusters of a unit from different sessions have a similar principal component composition and the same set of single units was recorded during the entire recording session.

A unit was regarded as CS-onset responsive neurons if the firing rate of 500ms following CS-onset was significantly different from the baseline (2s preceding the CS onset) ($p<0.01$, Wilcoxon

signed-rank test) (Beyeler et al., 2016). To investigate the unit responses, CS-evoked neural activities were normalized to the firing rates of 500ms preceding CS onset for each CS, except for units that did not exhibit any firing within this interval. Those neurons were normalized to the basal firing rates calculated from 10s, 30s preceding CS onset for each CS or all pre-CS intervals of the session. Z-score peri-stimulus time histograms (PSTHs) of averaged CS responses were constructed for each neuron and then averaged from 10 CSs.

Histology. At the end of the experiment, rats were anesthetized with urethane (1g/kg) by intraperitoneal injection, and electrolytic lesions were created by passing a current (10μA, 5-20s) through recording microwires to discrete the location of units. The duration of current injection was varied to determine the exact region where each unit was located. More prolonged current injections produced more extensive lesions. After the current injection, animals were transcardially perfused with 0.9% saline solution and 10% buffered formalin. Brains were removed and post-fixated overnight. Coronal sections (100μm thick) were obtained by using a vibroslicer (NVSL; World Precision Instruments) and stained with cresyl violet. The placement of the recording microwires was confirmed under a light microscope.

Statistical analysis. To compare the behavioral results among

training sessions, averaged data points were analyzed using a non-parametric Friedman test. The CS responsiveness of BA units was determined using a Wilcoxon signed-rank test comparing 500ms after CS-onset to baseline window of 2s. A difference of the mean Z-score value was compared with a non-parametric Friedman test (Beyeler et al., 2016).

Results

Appetitive and aversive behavior training alter reward port approach and defensive behavior

To figure out neural activity alteration within the BA followed by CS-related emotional value change, I used appetitive and aversive behavior training paradigm (see Material and Method) (Fig. 6A). Reward port approach to CS-R onset was measured by nose poking, and fear level to CS-F was measured by freezing. 2.8 kHz continuous tone (10s) CS was used for reward-related behavior, and the green LED (10s) was used for fear-related behavior. After last reward conditioning, rats showed robust port access level when reward-related cue (CS-R) was presented, and port access level decreased after reward extinction (Friedman test, ***$p<0.001$, ****$p<0.0001$) (Fig. 6B). After fear conditioning, rats showed freezing behavior when fear-related cue (CS-F) was presented and freezing diminished after fear extinction (Friedman test, ****$p<0.0001$) (Fig. 6C). This behavior modification indicated that rats altered CS-dependent value by conditioning and extinction behavior training.

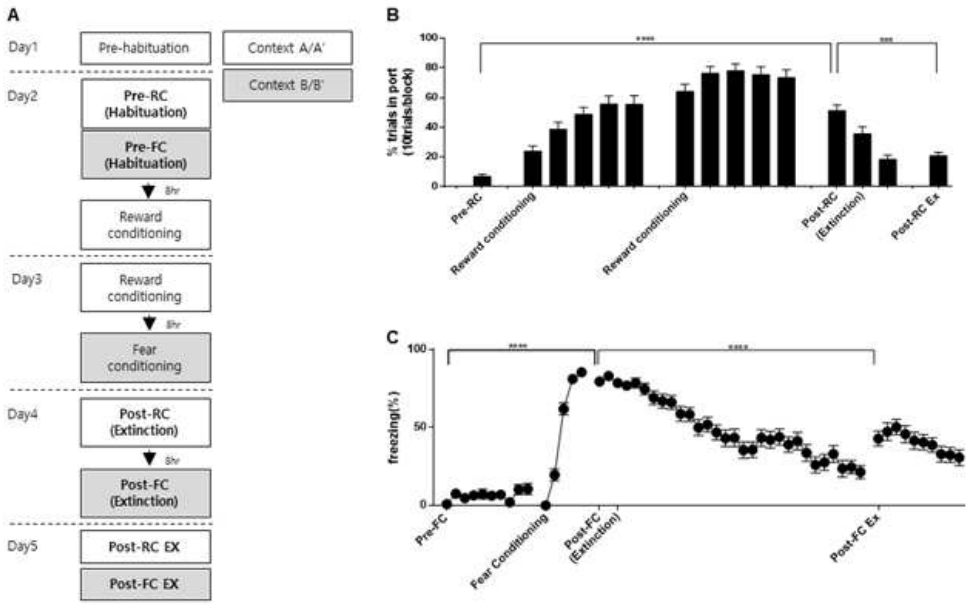

A
Day1
Pre-habituation
Context A/A'
Context B/B'
Day2
Pre-RC (Habituation)
Pre-FC (Habituation)
8hr
Reward conditioning
Day3
Reward conditioning
8hr
Fear conditioning
Day4
Post-RC (Extinction)
8hr
Post-FC (Extinction)
Day5
Post-RC EX
Post-FC EX
B
% trials in port (10trials/block)
Pre-RC
Reward conditioning
Reward conditioning
Post-RC (Extinction)
Post-RC Ex
C
freezing(%)
Pre-FC
Fear Conditioning
Post-FC (Extinction)
Post-FC Ex

Figure 6. Reward behavior alters reward port approach while fear behavior changes defensive behavior.

A. The behavior procedure used in the experiment. Habituation conducted 8 hours before first reward conditioning. Reward and fear conditioning was conducted on day 3. Reward and fear extinction was carried out 1day after the conditioning session. A single unit recording was conducted during day2-5. The white and grey shade represents a different context. **B.** The learning curve of the entire reward behavior session. Each block represents port access for 10 trials. Error bars indicate SEM. Rats, which accessed to the port for the first 5 trials during post-RC session regarded as the learner for reward conditioning. Learners used for behavior and neural analysis. ***$p<0.001$, ****$p<0.0001$. **C.** The learning curve of the entire fear behavior session. Error bars indicate SEM. ****$p<0.0001$.

Long-term single unit recording within BA

Location of neurons recorded from the electrode within the BA was confirmed by histological analysis (Fig. 7A). Neurons, which recorded stably during entire behavior training and verified by principal component analysis (see Material and Method) (Fig. 7B and C), were used in the data analysis process. After neural verification, we found CS-R and CS-F responsive neurons during entire behavior training sessions.

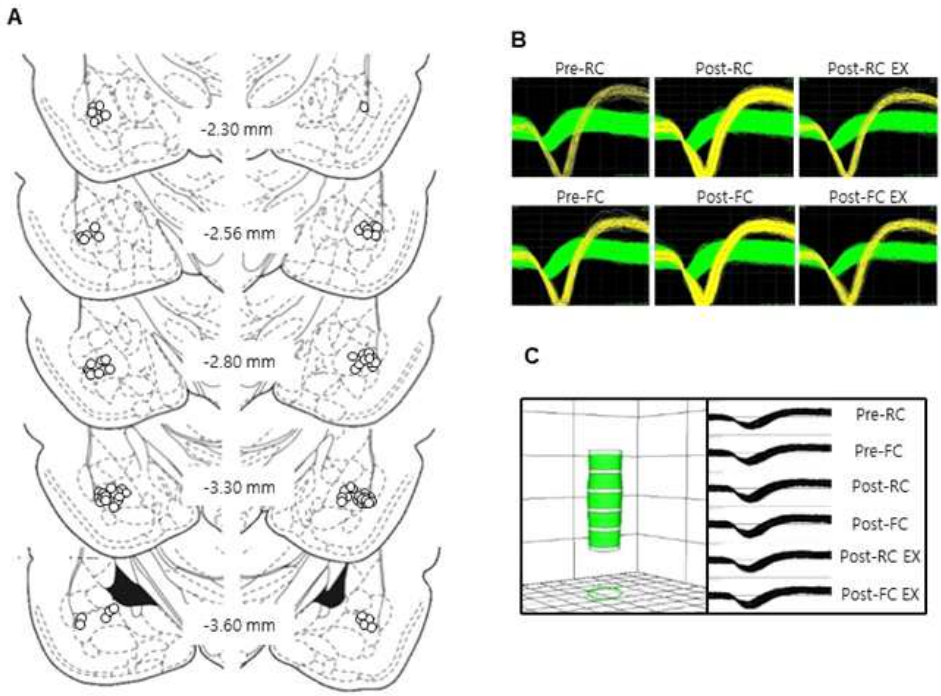


Figure 7. Representative data of long-term single unit recording within BA.

A. Histological verification of the electrode placements (n=43). **B.** Representative waveforms of BA neurons recorded from a single electrode. Neurons were stably observed throughout the entire reward and fear behavior training period. Grid: 55μV, 100μs. **C.** Verification of single units from long-term recordings using principal component space cylinders. A straight cylinder suggests that the same set of single units was recorded in different behavior session.

Appearance of the session-specific cue-onset response BA neurons after appetitive and aversive behavior training

During reward and fear behavior training sessions including habituation, 277 neurons were analyzed, and 62% (n=171) of the neurons showed CS-onset response based on the firing rate (see Material and Method). Learning-dependent CS-onset response neurons included neurons, which showed emerged CS-onset response (Herry et al., 2008) or enhanced excited or inhibited response after behavior training compared to habituation (Goosens et al., 2003; Grundemann and Luthi, 2015). 61% of CS-onset response neurons (n=104) exhibited neural activity changes followed by reward and fear behavior training and regarded as learning-dependent CS-onset responsive neurons (Fig. 8). Among them, 75% (n=78) exhibited CS-onset response to CS-R or CS-F, specifically. 16% of CS-onset responsive neurons (n=17) showed CS-onset response to both CS-R and CS-F (Fig. 8). From previous studies, neurons, which showed CS-R or CS-F specific response, classified as valence neurons (Beyeler et al., 2016; Paton et al., 2006). On the other hand, both CS-R and CS-F responsive neurons categorized arousal neurons (Shabel and Janak, 2009). Therefore, most of learning-dependent CS-onset responsive neurons after reward and fear behavior training was comprised of valence neurons. Moreover, valence neurons divided by single-session or multi-session response neurons. Single-session

response neurons showed neural response alteration after conditioning or extinction session specifically. On the other hand, multi-session response neurons exhibited neural activity modification after conditioning and maintained their response until the extinction session. 80% of valence neurons displayed a single-session response instead of multi-session (Fig. 8). As a result, the majority of CS-onset response neurons showed a session-specific response after cue-dependent value change by emotional learning.

Related to the reward behavior, single-session response neurons exhibited CS-R onset response after reward conditioning or extinction (Fig. 9A and B). On the other hand, multi-session response neurons, also known as extinction-resistance neurons (Goosens et al., 2003; Repa et al., 2001), were activated to CS-R onset after both reward conditioning and extinction (Fig. 9C). CS-F response neurons also showed a similar tendency. Related to the fear behavior, single-session response neurons displayed CS-F onset response after fear conditioning or extinction (Fig. 10A and B). Multi-session response neurons showed excited or inhibited neural activity after both fear conditioning and extinction (Fig. 10C). Mean z-score of learning-dependent CS-onset responsive neurons indicated neural response alteration. Single-session response CS-R related value coding neurons exhibited excited neural response after specific conditioning or extinction session. On the other hand, mean z-score of multi-session response neurons displayed constant neural activity alteration after both conditioning and extinction sessions (Only excited

neurons were included) (Friedman test, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001) (Fig. 11A). Similar neural alteration appeared to the mean z-score of CS-F related learning-dependent CS-onset response neurons. Single-session response CS-F related value coding neurons displayed session-specific excited neural response while multi-session response neurons maintained neural activity modification through conditioning to extinction session (Only excited neurons were included) (Friedman test, *p<0.05, **p<0.01) (Fig. 11B).

As a result of appetitive and aversive conditioning and extinction, 80% of valence neurons showed a single-session response while CS updated emotional information (Fig. 8). Because of a large proportion of learning-dependent CS-onset response neurons within the BA was comprised of single-session response valence neurons, I assumed that BA neurons represented cue specific value change by using separate neural population.

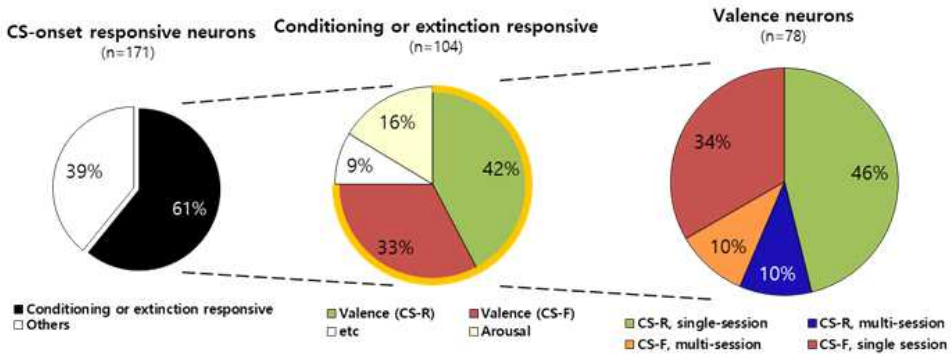


Figure 8. The distinct neural activity of CS-onset responsive neurons during reward and fear behavior training.

Percentage of valence and arousal neurons after reward and fear behavior training. CS-onset responsive neurons were found during the entire recording session including habituation. 62% of neurons (n=104) classified learning-dependent CS-onset responsive neurons within CS-onset responsive neurons. Valence neuron, which showed specific response to CS-R or CS-F, occupied 75% (n=78) of learning-dependent CS-onset responsive neurons. Among them, 80% (n=62) showed single-session response while 20% (n=16) displayed multi-session response.

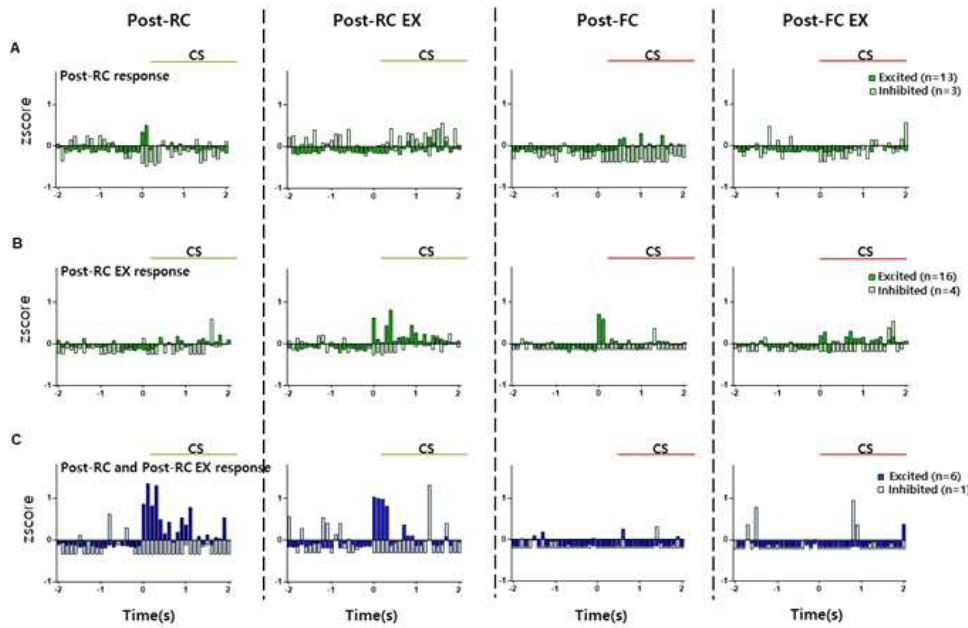


Figure 9. CS-onset response of CS-R related value coding neurons.

A, B. Z-score for single-session response CS-R neurons. A showed z-score for post-RC response neurons whereas B exhibited z-score for post-RC EX response neurons. **C.** Z-score for multi-session response CS-R neurons. Those neurons displayed CS-onset response to both post-RC and post-RC EX session.

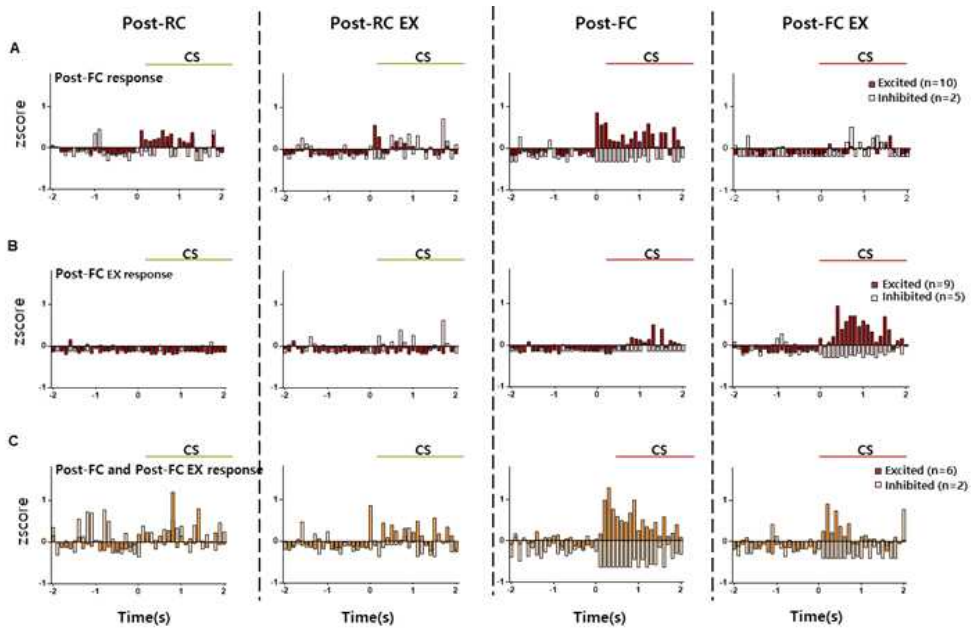


Figure 10. CS-onset response of CS-F related value coding neurons.

A, B. Z-score for single-session response CS-F neurons. A showed z-score for post-FC response neurons whereas B represented z-score for post-FC EX response neurons. **C.** Z-score for multi-session response CS-F neurons. Those neurons showed CS-onset response to both post-FC and post-FC EX session.

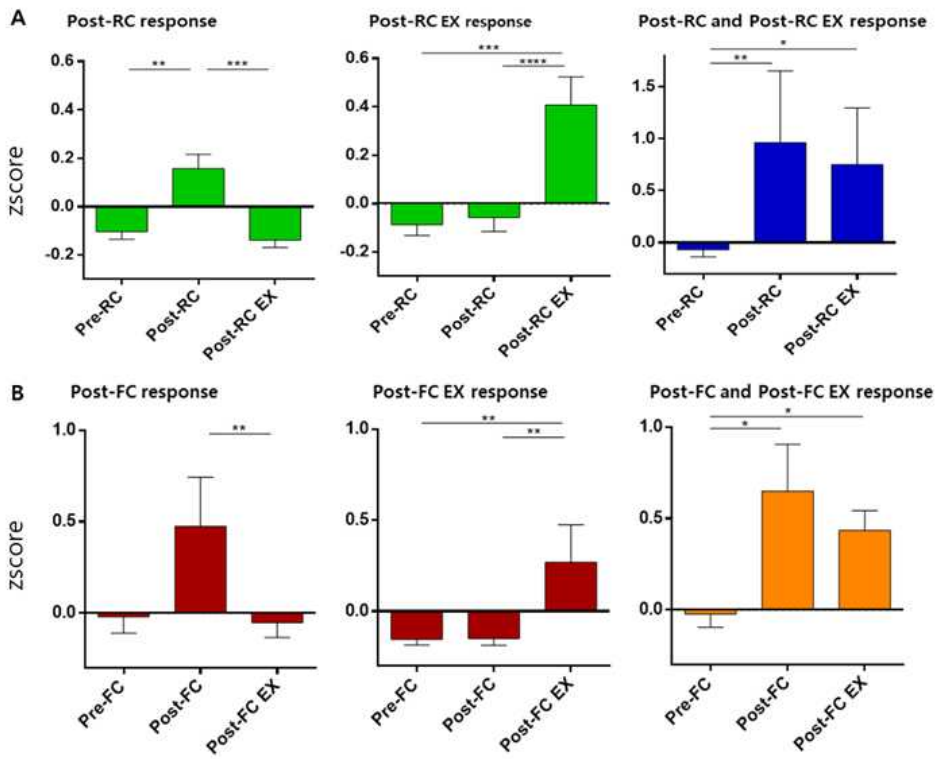

A
Post-RC response
Post-RC EX response
Post-RC and Post-RC EX response
zscore
Pre-RC
Post-RC
Post-RC EX
B
Post-FC response
Post-FC EX response
Post-FC and Post-FC EX response
zscore
Pre-FC
Post-FC
Post-FC EX

Figure 11. Mean z-score of CS-R and CS-F related value coding neurons.

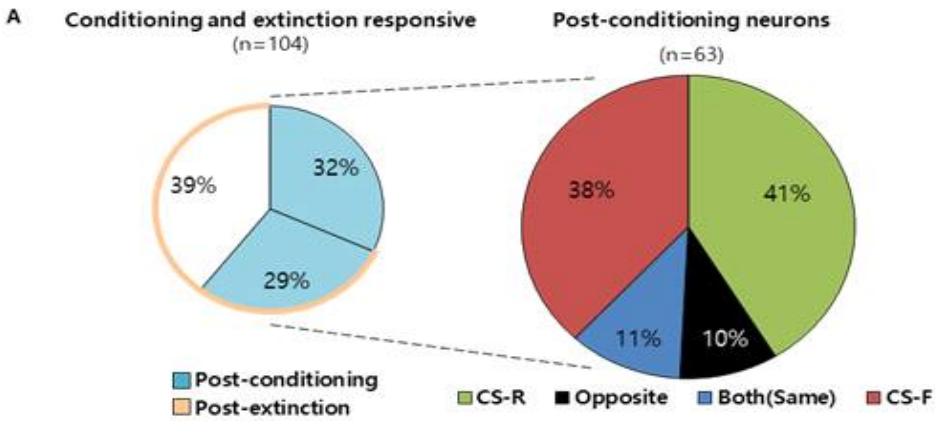
A. Mean z-score for excited single-session and multi-session CS-R response neurons. Single-session response neurons showed excited neural response during post-RC or post-RC EX, respectively. Multi-session response neurons displayed CS-onset response during both post-RC and post-RC EX session. *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$. **B.** Mean z-score for excited single-session and multi-session CS-F response neurons. Single-session response neurons altered neural response during post-FC or post-FC EX, respectively. Multi-session response neurons displayed CS-onset response during post-FC and post-FC EX session. *$p<0.05$, **$p<0.01$.

Proportion of valence neurons during post-conditioning and post-extinction session

Previous data indicated that a large proportion of session-specific response valence neurons emerged after each behavior training session. Next, I figured out the proportion of CS-R and CS-F responsive neurons during each post-conditioning and post-extinction session. By doing so, I confirmed that the proportion of valence and arousal neurons showed whether a similar tendency compared to the previous studies. Post-conditioning response neurons divided by CS-R or CS-F specific responsive neurons (CS-R, 41%, n=26; CS-F, 38%, n=24), which represented valence, and both CS-R and CS-F responsive neurons (Both (Same), 11%, n=7; Opposite, 10%, n=6), which represented arousal (Fig. 12A). Mean z-score indicated that valence neurons exhibited an excited or inhibited CS-onset response during post-RC or post-FC compared to other behavior sessions. On the other hand, arousal neurons showed excited or inhibited neural response during fear and reward conditioning sessions compared to the extinction sessions (Friedman test, $*p<0.05$, $**p<0.01$, $****p<0.0001$) (Fig. 13).

Post-extinction neurons, which displayed a novel or enhanced CS-onset response to CS-R or CS-F during the post-extinction session, were also found in the BA. Post-extinction neurons composed of CS-R or CS-F specific responsive neurons (CS-R, 45%, n=32; CS-F, 34%, n=24), which represented valence, and both CS-R

and CS-F responsive neurons (Both (Same), 15%, n=11; Opposite, 6%, n=4), which represented arousal (Fig. 12B). Mean z-score indicated that valence neurons displayed session-specific excited or inhibited CS-onset response during post-RC EX or post-FC EX compared to the other behavior sessions. In contrast, arousal neurons exhibited the tendency of excited or inhibited neural activity followed by both CS-R and CS-F during extinction sessions (Friedman test, *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$) (Fig. 14). These results showed increased valence neural population compared to the previous data (Beyeler et al., 2016; Shabel and Janak, 2009). Therefore, it also suggested that a different population of valence neurons appeared as a result of emotional learning and value alteration.


A
Conditioning and extinction responsive
(n=104)
Post-conditioning neurons
(n=63)
32%
39%
29%
38%
41%
11%
10%
Post-conditioning
Post-extinction
CS-R
Opposite
Both(Same)
CS-F

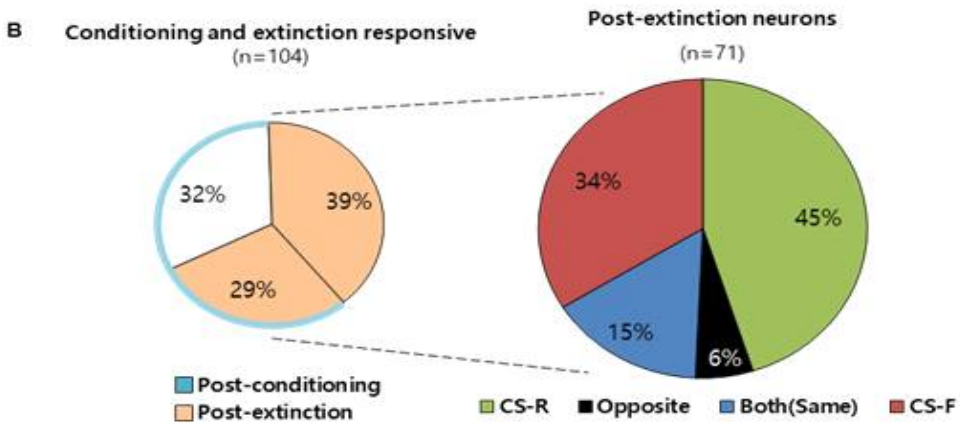

B
Conditioning and extinction responsive
(n=104)
Post-extinction neurons
(n=71)
32%
39%
29%
34%
45%
15%
6%
Post-conditioning
Post-extinction
CS-R
Opposite
Both(Same)
CS-F

Figure 12. Neural activity within BA during post-conditioning and post-extinction sessions.

A. Percentage of valence and arousal neurons during the post-conditioning session. Valence neurons composed CS-R (n=26, 41%) and CS-F response (n=24, 38%) neurons. Arousal neurons composed same (n=7, 11%) and opposite (n=6, 10%) response neurons. **B.** Percentage of valence and arousal neurons during the post-extinction session. Valence neurons composed CS-R (n=32, 45%) and CS-F response (n=24, 34%) neurons. Arousal neurons composed same (n=11, 15%) and opposite (n=4, 6%) response neurons.

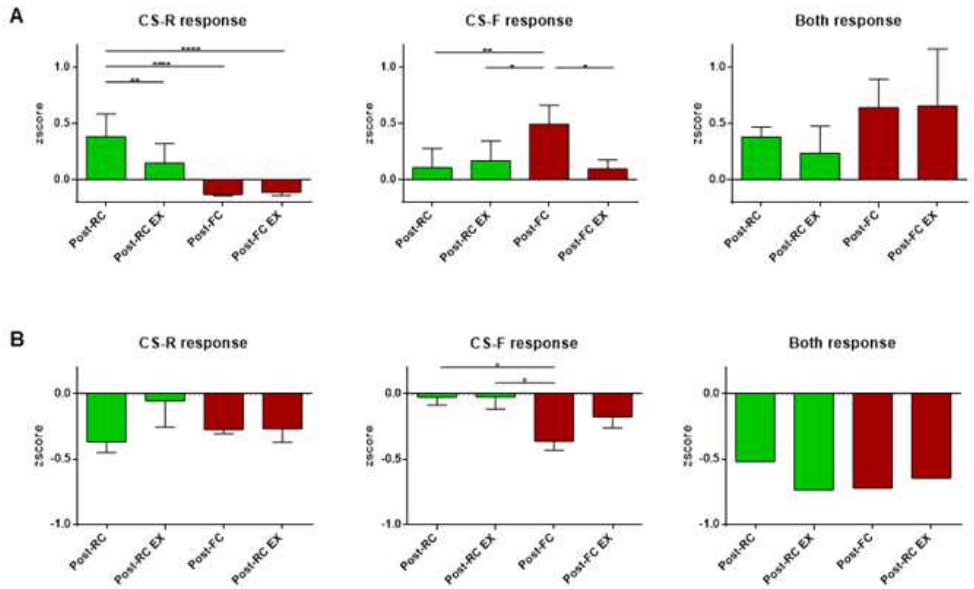


Figure 13. CS-onset response during post-conditioning session.

A. Mean z-score for excited CS-R, CS-F and both (same) response neurons during the post-conditioning session, respectively. *p<0.05, **p<0.01, ****p<0.0001. **B.** Mean z-score for inhibited CS-R, CS-F and both (same) response neurons during the post-conditioning session, respectively. *p<0.05, **p<0.01.

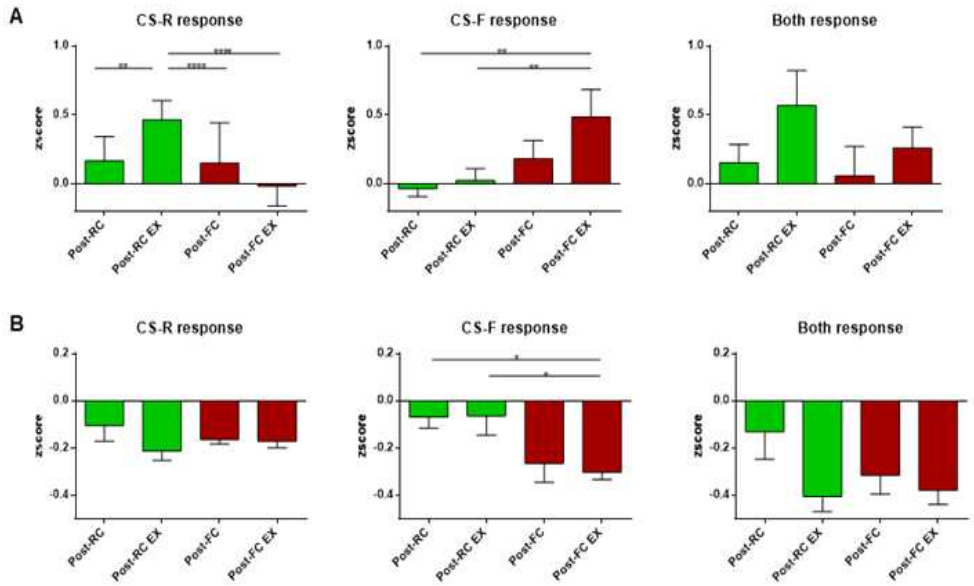


Figure 14. CS-onset response during post-extinction session.

A. Mean z-score for excited CS-R, CS-F and both (same) response neurons during the post-extinction session, respectively. *<0.05, ***p<0.001, ****p<0.0001. **B.** Mean z-score for inhibited CS-R, CS-F, and both (same) response neurons during the post-extinction session, respectively. *p<0.05, **p<0.01.

BA neural response to CS-onset before CS-dependent learning

Neurons within the BA showed CS-dependent response after emotional learning, but some of the neurons also exhibited intense response during the habituation period. CS-response neurons during habituation sessions also divided by two distinct population: CS-onset response during the habituation period only or neural response altered after habituation. Especially, neurons, which altered neural activity during the habituation period only or showed the most robust neural activity during habituation session defined as habituation-related neurons (Fig. 15). Mean z-score of CS-onset response neurons during habituation session exhibited excited or inhibited CS-R or CS-F response compared to the post-conditioning and post-extinction sessions (Friedman test, *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$) (Fig. 16A and B). Others, which defined as CS resistance neurons, presented constant neural response even after associative learning and extinction but their mean z-score diminished followed by fear and reward behavior process (Friedman test, *$p<0.05$, **$p<0.01$, ****$p<0.0001$) (Fig. 16C and D).

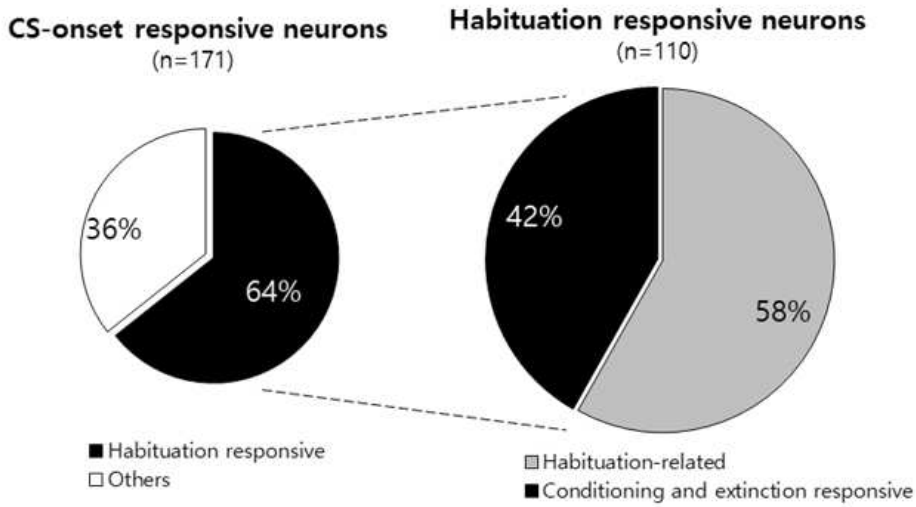


Figure 15. Part of the CS-onset response neurons showed CS-onset response during habituation session.

64% of CS-onset responsive neurons showed CS-onset response during habituation (n=110). 42% of CS-onset responsive neurons exhibited enhanced excited or inhibited response after reward and fear behavior training (n=46). 58% neurons, which disappeared or showed smaller CS-onset response after behavior training, classified as habituation-related response neurons (n=64). Neurons which showed enhanced CS-onset response after behavior training included learning dependent CS-onset responsive neurons.

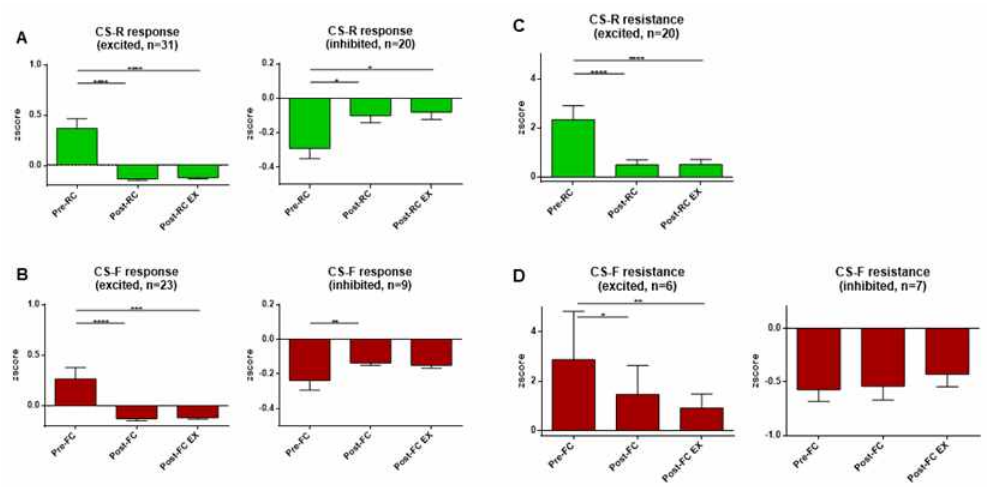


Figure 16. Mean CS-onset response of habituation session response neurons.

A, B. Mean z-score for excited and inhibited CS-R, CS-F response neurons during habituation session, respectively. *p<0.05, ****P<0.0001. **C, D.** Mean z-score of excited and inhibited CS-R, CS-F resistance neurons during habituation session, respectively. CS-R and CS-F resistance neurons maintained neural response after associative learning but their neural activity diminished or changed. *p<0.05, **p<0.01, ***p<0.001, ***p<0.0001.

Discussion

In the study, I investigate the role of the BA while cue-dependent value modification. By tracking down the neurons during entire behavior session, including conditioning and extinction, I observed the existence of CS-onset response neurons, which specifically alter their activity followed by behavior task. Furthermore, those neurons exhibited a single-session response instead of multi-session (Fig. 8). These results indicate that a novel population appears within the BA when the cue-dependent emotional value is changed. Therefore, the BA update the value of the CS through the distinct neural population.

Previous studies indicate that a separate group of cue specific responsive neurons exist within the BLA after various emotional associative learning is conducted with a different cue (Lee et al., 2017; Sangha et al., 2013). Moreover, other experimental results suggest that the cue specific responsive neurons represent valence (Beyeler et al., 2016; Namburi et al., 2015; Paton et al., 2006) instead diverse cue response neurons indicate arousal (Shabel and Janak, 2009). Furthermore, a recent study demonstrated that population activity alteration appeared as a result of cue-dependent value updating by reversal learning (Zhang and Li, 2018). However, this study investigated the population activity of the BLA during the reversal learning process. Even though a previous study (Sangha,

2015) showed part of safety cue responsive neurons activated by fear cue after fear extinction and suggested similar information encoded by same neural population, it could not explain the role of newly appeared fear cue response neurons. Therefore, the role of the BA during cue-dependent value change as a single cell level is unclear. By discovering the existence of session-specific responsive neural populations followed by emotional learning and value change, I consider cue-specific responsive neurons play a pivotal role in updating the emotional value.

BA neurons showed CS-onset response even before CS-dependent emotional learning (Fig. 15). Neurons, which reduce or change their CS-onset response after emotional associative learning, are possible to represent new sensory cue-dependent response because the expectation to the same cue can diminish neural activity (Belova et al., 2007). Other population of neurons, which activate habituation session only, are explained as BA neural representation of cue-related value information when animals confront new external stimuli. If those neural populations respond to sensory stimuli instead of emotional information, the neural response should exist after cue-dependent learning. Together, it suggests that neurons within the BA represent the current emotional value of the specific cue.

Recent studies report that ensemble activity of BA neurons is crucial to represent emotional memory (Grewe et al., 2017; Kyriazi et al., 2018; Rozeske et al., 2018). At the same time, other studies

indicate that part of the BA neurons is activated as a result of emotional memory formation and stimulating those neurons recall the memory (Han et al., 2007; Kim et al., 2016; Liu et al., 2012; Livneh and Paz, 2012; Yiu et al., 2014). Moreover, the distinct neural population is used to represent CS-dependent emotional memory even CS represents the same valence (Cai et al., 2016; Rashid et al., 2016). I also figure out that the proportion of the valence neurons, which is discovered by tracking down the neural activity within the entire behavior sessions, is increased compared to the previous studies (Fig. 12) (Beyeler et al., 2016; Shabel and Janak, 2009). Together, cue specific response neurons after emotional learning have a distinct role when animals encode cue-dependent information even though population activity is also related to representing emotional memory.

In this study, I regard extinction as a cue-dependent value alteration. The view of the extinction during valence coding process is uncertain, but recent research suggests that opposing value requires for the extinction process (Felsenberg et al., 2018). Therefore, cue-dependent value change due to the extinction is regarded as a value modification.

As a result, experimental data demonstrate that most of the CS-onset responsive neurons after emotional associative learning exhibit session-specific activity when the learning-dependent cue is presented. From this data, I suggest that the segregated neural population within the BA represents emotional information and related

value change. Therefore, BA neurons update a current emotional value of the stimuli through the independent neural population.

Chapter 2.

Basal amygdala (BA) encodes emotional event by using independent neural populations

Abstract

During the emotional memory process, distinct neural activity appears to represent valence information. However, the experiments about valence encoding use associative learning, which also recruits a different neural population. Therefore, the role of the population activity within the basolateral amygdala (BLA) is uncertain. Moreover, those experiments investigate neural activity within the BLA even though lateral (LA) and basal (BA) amygdala, which composed of BLA, have different anatomical and physiological properties. Because of the previous studies indicate that the LA show stable neural response during associative learning, I focus on the BA to investigate the dynamic neural activity during the emotional learning process.

To examine whether the dynamic neural activity within the BA encodes emotion-involved event, I conduct two different experiments: repeated same fear conditioning and consecutive retrievals after single fear conditioning with two different intervals. As a result, I figure out that 86% of learning-dependent CS-onset response neurons show conditioning session-specific response. Furthermore, to ensure the appearance of the distinct neural population within the BA is an outcome of the conditioning event, I conduct consecutive retrievals with two different intervals. Interestingly, almost 70% of the neural population also exhibits

retrieval-session specific response followed by repeated retrieval regardless of the time interval between retrieval sessions. Moreover, as control of the BA neural activity, I collect LA neurons during repeated retrieval with 1hour intervals and LA neurons display a more stable response compared to the BA. Altogether, these data indicate that BA represents emotional state via distinct neural population.

Key words: Basal amygdala. valence, associative learning, event-specificity

Introduction

Amygdala is a brain structure which encodes emotional memory. Because the animals have to deal with the environmental stimuli properly for survival, it is essential to associate emotional value and external stimuli. Related to the emotional memory process, lots of evidence indicate that activation or inactivation of the amygdala alter emotion-related behaviors (Felix-Ortiz et al., 2013; Hatfield et al., 1996; Nader et al., 2001; Namburi et al., 2015; Phelps and LeDoux, 2005) and neural response appears after emotional learning (Herry et al., 2008; Quirk et al., 1995; Tye et al., 2008). .

Previous studies demonstrate that valence information is encoded by distinct neural population within the basolateral amygdala (BLA) (Beyeler et al., 2016; Paton et al., 2006). Furthermore, recent studies show that the stimulating valence coding population within the BLA induces behavior outputs (Gore et al., 2015; Namburi et al., 2015; Stuber et al., 2011). However, electrophysiological experiments about valence coding use both positive and negative associative learning (Beyeler et al., 2016; Namburi et al., 2015; Paton et al., 2006; Shabel and Janak, 2009). Associative learning also recruits discerned neural population such as fear and fear extinction neurons (Herry et al., 2008). Moreover, different neural circuits are recruited when fear retrieval occurs at different time points (Do-Monte et al., 2015; Do Monte et al., 2016). Therefore, even though amygdala recruits distinct

neural population continually in the process of emotional learning, the role of the dynamic neural activity within the BLA is uncertain.

Most of the valence-related experiment focuses on the BLA. The BLA composed of lateral (LA) and basal (BA) part of the amygdala and each of these structures has different anatomical and physiological properties (McDonald, 1998). Furthermore, the LA show stable neural response when the same emotional event occurred (An et al., 2012; Han et al., 2009; Reijmers et al., 2007). Moreover, stimulating fear engram neurons within LA cause freezing response (Josselyn et al., 2015; Rashid et al., 2016). Because of the dynamic neural response appeared within amygdala during value coding process and the neural activity within BA is undiscovered, I assume that the BA has a different role compared to the LA.

In this study, I try to figure out the role of dynamic neural activity within the BA, whether they encode emotional events. By doing so, I conduct repeated fear conditioning, which is recognized as a different event with the same value. Moreover, to prove two identical conditioning events cause neural activity modification, I also conduct single fear conditioning with two different intervals. By tracking down neural activity within the BA, I observe neural response modification during entire behavior sessions.

Materials and Methods

Animals. Male Long-evans rats (n=47, 8 weeks old) were individually housed for 7-11d before experiments under an inverted 12h light/dark cycle (light off at 9:00 a.m.). Food and water were offered ad libitum. Animals were handled a day before the behavior. Behavior training was conducted during the dark cycle. Entire procedures were approved by the Institute of Laboratory Animal Resources of Seoul National University.

Behavior apparatus and procedures. Fear conditioning was conducted in a rectangular context with the stainless steel bar floor (Context A). Fear retrieval was performed in a circular context with the flat floor (Context B). Context A was a rectangular Plexiglas box (27 cm length x 26.5cm width x 30cm height) which was illuminated with white light and cleaned with 70% ethanol solution. Metal grid-connected with the electrical current source (Coulbourn Instruments). Context B was a Plexiglas cylindrical chamber (27cm length x 27cm width x 30cm height) which was illuminated with red light and cleaned with water and sprayed 1% acetic acid. Both contexts were located in a sound-attenuating chamber.

Rats were habituated on day 1. Animals were placed in context B for 10min twice a day, first only exposed to the context

and later with five presentations of the CS. 2.8 kHz tone pips (200ms duration repeated at 0.9 Hz, 85dB), which compose 27 tone pips were used as CS for fear conditioning and retrieval.

Double fear conditioning experiments, rats were given three presentations of the tone CS (inter-trial interval; 140s) in context B (Pre-conditioning) on day 2. 8 hours later, fear conditioning was conducted within context A by pairing the CS with an electric foot shock (0.5mA, 0.5s, 5 CS/US pairings, inter-trial interval; 90s) co-initiating with the onset of the last tone pip. On day 3, the first retrieval was conducted (Post-conditioning 1), and second fear conditioning was performed 8 hours later. On day 4, rats were given three presentations of the tone CS in context B (Post-conditioning 2). The animals, which showed increased freezing response during averaging 3 tone CS presentations after fear conditioning, were used for the data analysis.

Single fear conditioning experiments, rats were given three presentations of the tone CS in context B (Pre-conditioning) on day 2. 8 hours later, fear conditioning was conducted within context A by pairing the CS with a strong electrical foot shock (0.7mA, 0.5s, 7 CS/US pairings, inter-trial interval; 90s) co-initiating with the onset of the last tone pip. On day 3, the first retrieval was conducted, which represent three tones CS in context B (Post-conditioning 1). The second retrieval performed on day 4 for a long-term interval test while the second retrieval was conducted an hour later for a

short-term interval test (Post-conditioning 2). The animals, which exhibited increased freezing response during 3 tones CS presentations, were used for the data analysis. All of the behavior training sessions were videotaped. Trained observers quantified conditioned freezing. The animals were regarded to be freezing when there was no movement except for respiratory activity for 1s during 27 tone pip CS presentation. The total freezing time normalized to the duration of the CS presentation (An et al., 2012; Kim et al., 2010).

Surgery. Rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50mg/kg) and maintained with isoflurane (1-1.5%) in O_2. Rats were mounted on a stereotaxic apparatus (Storting) and bilaterally implanted with fixed-wire electrodes targeted to the BA (anterior-posterior; -2.70mm, medial-lateral; ±5.25mm and dorsal-ventral; -9.10mm). The electrodes consisted of eight insulated nichrome microwires (50μm outer diameter, impedance 0.2-0.5 MΩ; California Fine Wire), which was contained in a 21 gauge stainless steel guide cannula. The electrodes were affixed to the skull with dental cement (Vertex). After surgery, analgesic (Metacam; Boehringer) and antibiotics (KOCHA CETIO) were injected. Rats were allowed to recover for 7-11d.

In vivo single-unit recording. The neural response was acquired and analyzed using a Plexon MAP system (An et al., 2012; Herry et

al., 2008). Unit discrimination was conducted using Offline Sorter (Plexon). All waveforms were presented in a principal component space and clusters consisting of similar waveforms distinct from other clusters in principal component space was considered to be generated from a single neuron. Single-channel recordings were sufficient to discern single-unit responses due to the low neuronal density of the amygdala (Pare et al., 2004; Quirk et al., 1997). The long-term stability of single-unit isolation was determined by using Wavetracker (Plexon), which represented the principal component space cylinder of a unit recorded from different sessions (An et al., 2012; Herry et al., 2008; Tseng et al., 2011). A straight cylinder indicates that the clusters of the unit have a similar principal component composition and the same set of single units was recorded during the entire training session.

A unit was regarded as CS-onset responsive neurons if the firing rate of 500ms following CS-onset was significantly different from the baseline (2s preceding the CS onset) ($p<0.01$, Wilcoxon signed-rank test) (Beyeler et al., 2016). CS-evoked neural activities were normalized to the firing rates of 2s preceding CS onset for each CS, except for units that did not exhibit any firing within this interval. Neural activity of those neurons was normalized to the basal firing rates calculated from 10s, 30s preceding CS onset for each CS or all pre-CS intervals of the session. Z-score peri-stimulus time histogram (PSTHs) of averaged CS responses were constructed for each neuron and then averaged from 3 CSs.

Histology. At the end of the experiment, rats were anesthetized with urethane (1g/kg) by intraperitoneal injection, and electrolytic lesions were created by passing a current (10μA, 5-20s) through recording microwires. The duration of current injection was different among recording microwires to determine the exact region where each unit was located. Then, animals were transcardially perfused with 0.9% saline solution and 10% buffered formalin. Brains were removed and post-fixated overnight. Coronal sections (100μm thick) were obtained by using a vibroslicer (NVSL; World Precision Instruments) and stained with cresyl violet. The placement of the recording microwires was determined under a light microscope.

Statistical analysis. To compare the behavior results among training sessions, averaged freezing data for three tones CS were analyzed using a non-parametric Friedman test. The CS responsiveness of BA units was determined using a Wilcoxon signed-rank test comparing 500ms after CS-onset to baseline window of 2s. A difference of the mean Z-score value was compared with a non-parametric Friedman test (Beyeler et al., 2016).

Results

Repeated fear conditioning induces freezing response

To figure out whether BA neural activity alteration occurred as an emotional event-specific manner, I conducted the same CS-dependent fear conditioning twice with 1day interval (see Material and Method) (Fig. 17A). Freezing response to the CS-onset was regarded as a fear level. Weak fear conditioning protocol was used to ensure rats recognize each fear conditioning. After the first fear conditioning (post-FC1), rats showed enhanced freezing response compared to the pre-conditioning sessions (pre-FC). Rats exhibited gradually increased freezing response after second fear conditioning (post-FC2) compared to the pre-FC and post-FC (Friedman test, $*p<0.05$, $****p<0.0001$) (Fig. 17B and C). This results indicated that rats recognized each fear conditioning.

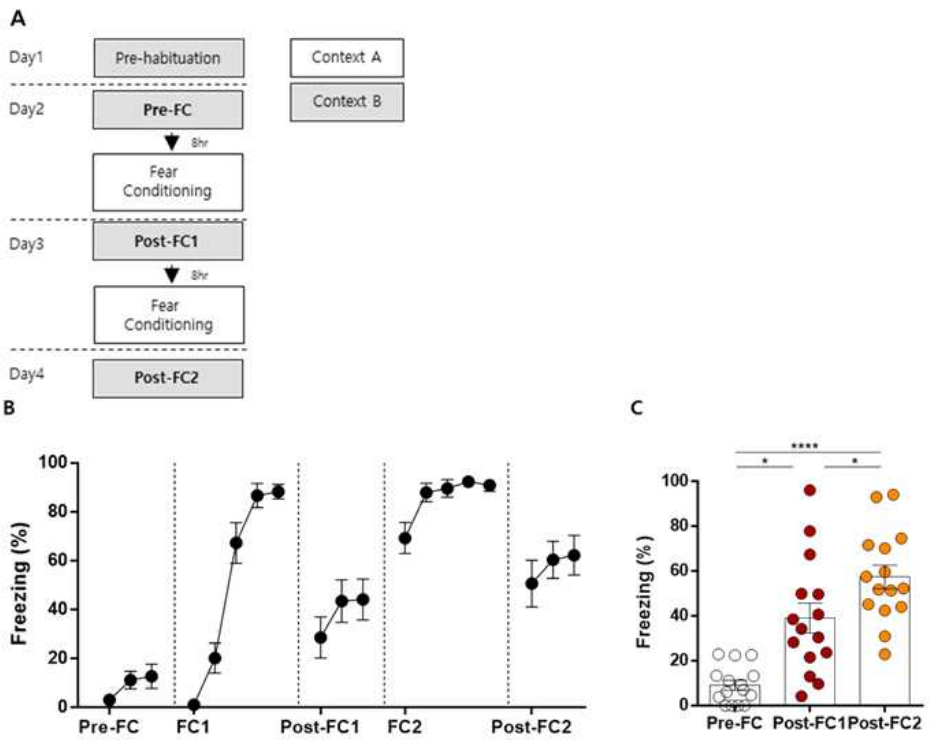

A
Day1
Pre-habituation
Context A
Context B
Day2
Pre-FC
8hr
Fear Conditioning
Day3
Post-FC1
8hr
Fear Conditioning
Day4
Post-FC2
B
Freezing (%)
100
80
60
40
20
0
Pre-FC
FC1
Post-FC1
FC2
Post-FC2
C
****
*
*
Freezing (%)
100
80
60
40
20
0
Pre-FC
Post-FC1
Post-FC2

Figure 17. Repeated fear conditioning induces elevated freezing response.

A. The behavior procedure used in the experiment. Habituation (pre-FC) was conducted 8 hours before first fear conditioning. On day 3, second fear conditioning was conducted followed by first retrieval (post-FC1). Day after second fear conditioning, the second retrieval was carried out to confirm freezing response alteration (post-FC2). Single unit activity was recorded during day 2-4. The white and grey shade indicate different context. **B.** The learning curve of the double fear conditioning session (n=15). Error bars indicate SEM. **C.** Mean freezing response during pre-FC, post-FC1, and post-FC2. Freezing response increased after fear conditioning. Error bars indicate SEM. $*p<0.05$, $****p<0.0001$.

Histological verification and representative data of long-term single-unit recording

The placement of the electrode within the BA was confirmed by histological analysis. These data included the histological location of the electrode, which was used during the repeated fear conditioning and repeated retrieval experiments (Fig. 18A). Histologically confirmed and stably recorded neurons throughout the entire behavior sessions, verified by principal component analysis (see Material and Method), were used in the data analysis (Fig. 18B and C). By using identified neurons, I analyzed neural activity before and after fear conditioning sessions.

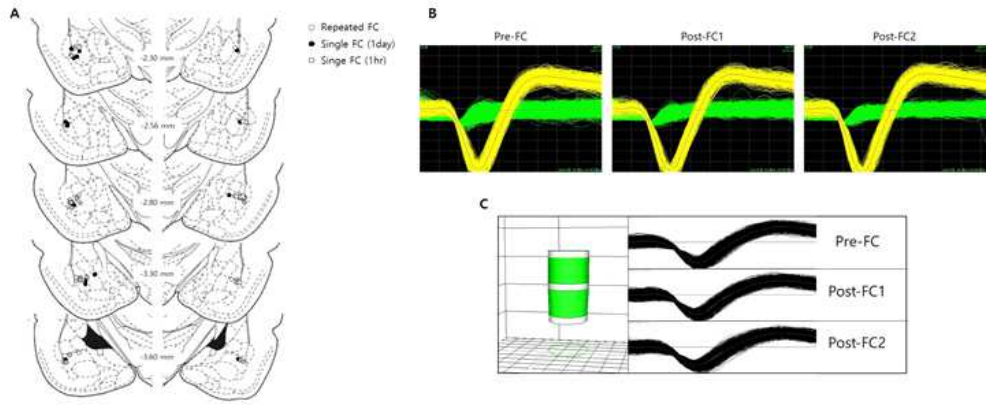


Figure 18. Histological verification and representative data of long-term single unit recording.

A. Histological verification of the electrode placement (n=47). The white circle indicates electrode placement which used in repeated fear conditioning behavior. Black and grey circle indicate electrode placement which used in repeated retrieval with 1day and 1hour interval, respectively. **B.** Representative waveforms of BA neurons recorded from a single electrode. Neurons stably appeared during the entire behavior sessions. Grid: 55μV, 100μs. **C.** Verification of single units from long-term single unit recording using principal component space cylinders. A straight cylinder indicated that same single unit was recorded throughout the entire behavior sessions.

Existence of discerned neural population after repeated fear conditioning

Throughout the entire behavior training, including pre-FC, I verified 112 neurons within the BA and 45% (n=50) neurons showed CS-onset response based on the firing rate alteration (see Material and Method). Conditioning-dependent CS-onset response neurons included both newly activated neurons and enhanced excited or inhibited neurons compared to the pre-FC session. Followed these criteria, 56% (n=28) of CS-onset response neurons regarded as conditioning responsive neurons (Fig. 19). 86% (n=24) of conditioning responsive neurons showed CS-onset response to post-FC1 or post-FC2 while remaining 14% (n=4) of the neurons exhibited CS-onset response to the both post-FC1 and post-FC2 sessions (Fig. 19). As a result, the majority of the learning-dependent CS-onset response neurons showed fear event-specific response even though rats went through the same fear conditioning.

Event-specific response neurons altered neural response after first or second conditioning. Post-FC1 response neurons showed enhanced neural activity within a post-FC1 session only while post-FC2 response neurons exhibited excited neural response within a post-FC2 session (Fig. 20A and B). On the other hand, non-event specific response neurons exhibited neural response alteration after first fear conditioning and maintained neural activity until the post-FC2 session (Fig. 20C). Mean z-score indicated that

event-specific response neurons - post-FC1 and post-FC2 response neurons - showed an excited neural response during post-FC1 or post-FC2, respectively. Non-event specific response neurons - both response neurons - showed a tendency of the increased neural response after first fear conditioning (Only excited neurons were included) (Friedman test, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$) (Fig. 21).

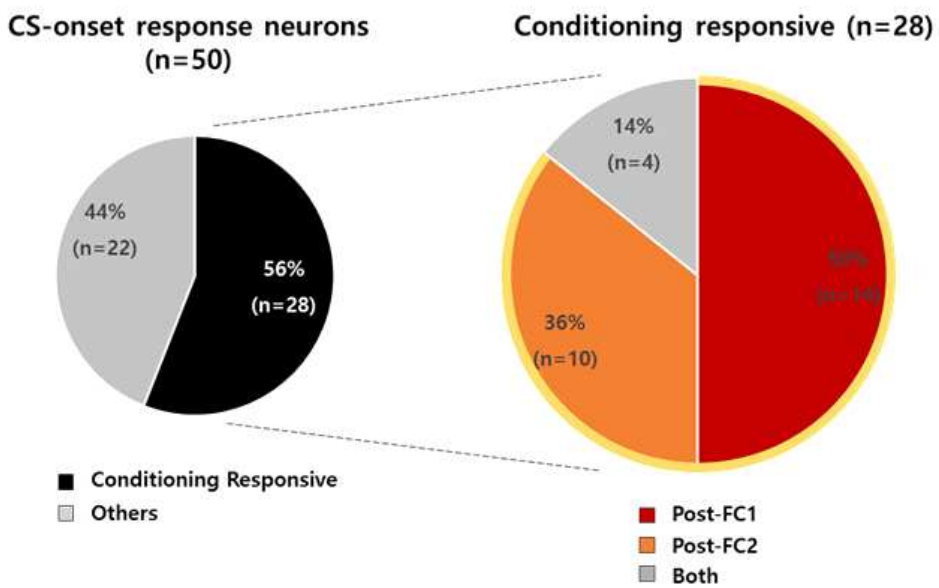


Figure 19. The appearance of the discerned neural population as a result of repeated identical fear conditioning.

Percentage of CS-onset response neurons after each fear conditioning. Neurons, which showed CS-onset response within the entire behavior session, regarded as CS-onset response neurons. 56% of CS-onset response neurons (n=28) classified conditioning-dependent CS-onset responsive neurons. Within conditioning-dependent CS-onset responsive neurons, 86& (n=24) showed session-specific response while 14% (n=4) neural response alteration after both first and second fear conditioning. Yellow boundary indicates session-specific response neurons.

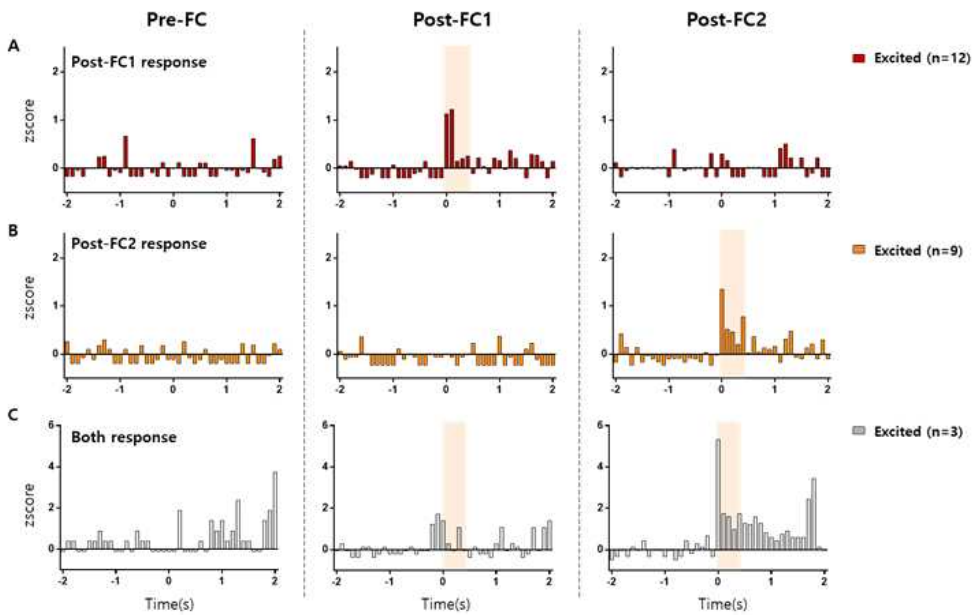


Figure 20. BA neural response after repeated fear conditioning.

A, B. Z-score for session-specific CS-onset response neurons. A represented z-score for post-FC1 response neurons while B indicated post-FC2 response neurons. **C.** Z-score for CS-onset response neurons, which exhibited CS-onset response after both first and second fear conditioning. Each graph included excited neurons only.

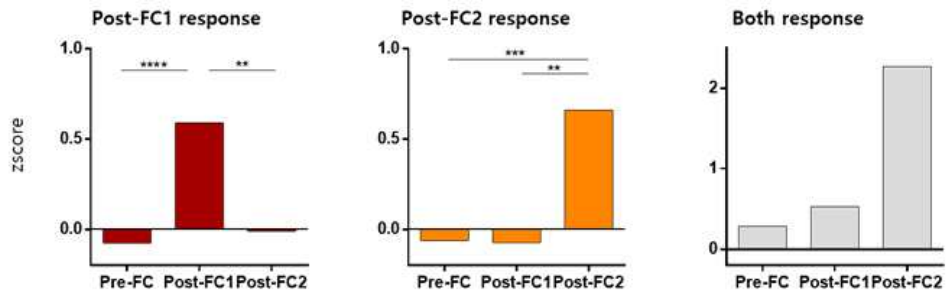


Figure 21. Mean z-score of cue-onset response neurons after repeated fear conditioning.

Mean z-score for excited session-specific CS-onset response neurons and both session response neurons. Session-specific CS-onset response neurons showed excited neural response either post-FC1 or post-FC2, respectively. Both session response neurons exhibited CS-onset response to both post-FC1 and post-FC2. **p<0.01, ***p<0.001, ****p<0.0001.

Fear response maintained during repeated retrieval with different intervals

Neural response during repeated fear conditioning indicated that the BA encodes CS-dependent emotional event as an event-specific manner by using distinct neural population whether the value of the CS was different or not. To confirm the independent neural population appeared as a result of an emotional event, I carried out consecutive two retrieval sessions with a different interval after single fear conditioning and recorded BA neurons during entire behavior training (Fig. 22A and 23A). Defensive behavior altered followed by fear conditioning and freezing response sustained until the end of the second retrieval session (Friedman test, ***$p<0.001$, ****$p<0.0001$) (Fig. 22B, C, and 23B, C). This behavior alteration demonstrated that rats learned CS-dependent fear after fear conditioning and extinction did not occur.

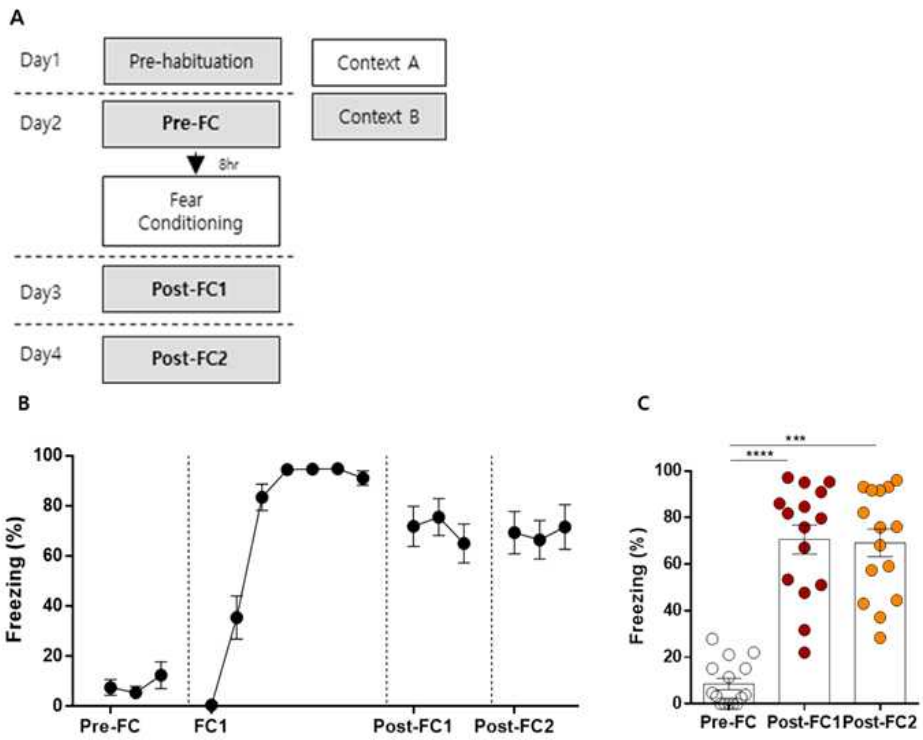

A
Day1
Pre-habituation
Context A
Day2
Pre-FC
Context B
8hr
Fear
Conditioning
Day3
Post-FC1
Day4
Post-FC2
B
Freezing (%)
100
80
60
40
20
0
Pre-FC
FC1
Post-FC1
Post-FC2
C
***
****
Freezing (%)
100
80
60
40
20
0
Pre-FC
Post-FC1
Post-FC2

Figure 22. Defensive behavior maintained during repeated retrieval with 1day interval after fear conditioning.

A. The behavior protocol used in the experiment. Habituation (pre-FC) was conducted 8 hours before the fear conditioning. First and second retrieval was carried out on day 3 and day 4, respectively. A single unit recording was conducted on day 2-4. The white and grey shadow indicate different context. **B.** The learning curve of the entire behavior sessions (n=15). Freezing response elevated after fear conditioning and similar freezing response appeared during the second retrieval session (post-FC2). Error bars indicate SEM. **C.** Mean freezing response before and after fear conditioning. Error bars indicate SEM. ***$p<0.001$, ****$p<0.0001$.

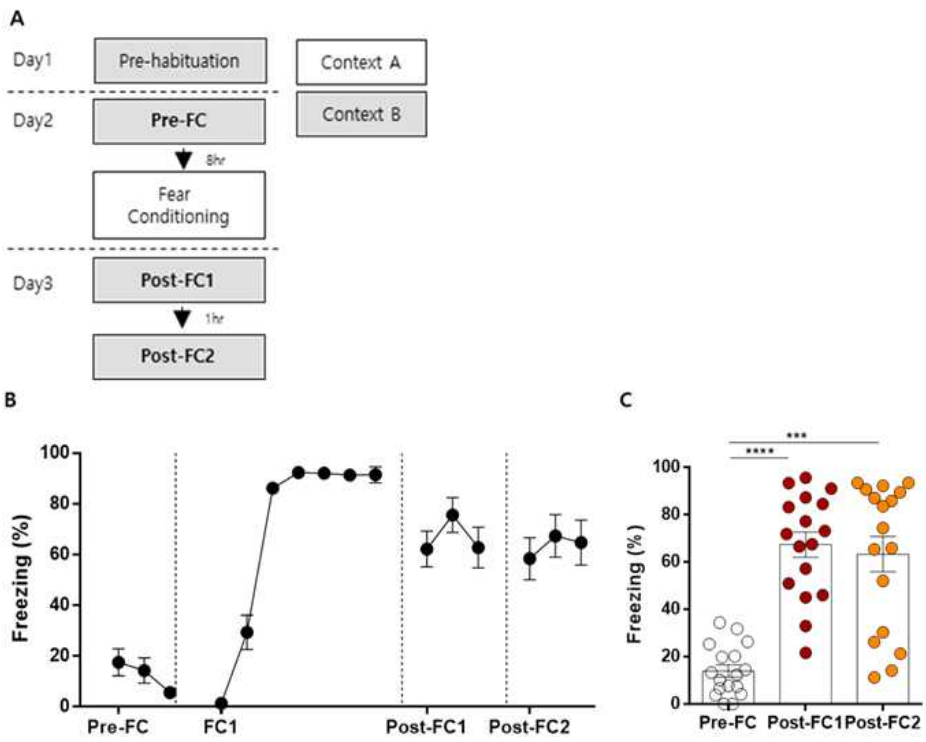

A
Day1
Pre-habituation
Context A
Day2
Pre-FC
Context B
8hr
Fear
Conditioning
Day3
Post-FC1
1hr
Post-FC2
B
Freezing (%)
100
80
60
40
20
0
Pre-FC
FC1
Post-FC1
Post-FC2
C
***
****
Freezing (%)
100
80
60
40
20
0
Pre-FC
Post-FC1
Post-FC2

Figure 23. The sustained freezing response appeared during repeated retrieval with 1hour interval after fear conditioning.

A. The behavior procedure used in the experiment. Habituation (pre-FC) conducted 8 hours before the fear conditioning. On day 3, two consecutive retrievals were carried out with 1hour interval. BA neural activity was recorded during day 2-3. The white and grey shadow indicate different context. **B.** The learning curve of the entire behavior sessions (n=17). Rats showed increased freezing response as a result of fear conditioning. Freezing response maintained until post-FC2. Error bars indicate SEM. **C.** Mean freezing response during pre and post-conditioning period. Error bars indicate SEM. ***$p<0.001$, ****$p<0.0001$.

Population activity of BA neurons during repeated retrieval after single fear conditioning

During two consecutive retrieval session with 1day interval, 96 neurons were analyzed. Among them, 42% (n=40) of the neurons regarded as CS-onset response neurons, including pre-FC based on the firing rate modification (see Material and Method). Same as repeated fear conditioning analysis, conditioning dependent CS-onset response neurons included neurons, which showed emerged neural response or enhanced neural response compared to the pre-FC. Followed by the criteria, 57% (n=23) of the CS-onset response neurons classified as conditioning-dependent response neurons. Interestingly, 70% (n=16) of the conditioning responsive neurons exhibited retrieval session-specific response (Fig. 24).

Retrieval session-specific response neurons displayed a specific response to the CS-onset during first (post-FC1) or second (post-FC2) retrieval session (Fig. 25A and B). On the other hand, neurons, which showed sustained neural response after fear conditioning, exhibited neural response alteration both post-FC1 and post-FC2 sessions compared to pre-FC (Fig. 25C). Mean z-score of the conditioning responsive neurons confirmed that session-specific responsive neurons reinforced their neural response at post-FC1 or post-FC2 specifically while non-session specific response neurons showed enhanced neural response during entire retrieval sessions (Only excited neurons were included) (Friedman test, $*p<0.05$,

**p<0.01) (Fig. 25D).

During repeated retrieval with 1hour interval, 115 neurons were analyzed and 35% (n=40) neurons showed CS-onset response, including pre-FC. 67% (n=27) of the CS-onset response neurons defined as conditioning-dependent CS-onset responsive neurons, followed by the same criteria about conditioning responsive neurons. Similar to the repeated retrieval with 1day interval, 78% (n=21) of the conditioning responsive neurons presented retrieval session-specific response, whereas the remaining 22% showed non-session specific response (Fig. 26).

Same as previous results, retrieval session-specific response neurons displayed CS-onset response during post-FC1 or post-FC2 session (Fig. 27A and B). On the other hand, neurons, which showed sustained neural response after fear conditioning, exhibited neural response alteration both post-FC1 and post-FC2 sessions compared to pre-FC (Fig. 27C). Mean z-score of the conditioning responsive neurons indicated that session-specific responsive neurons enhanced neural response at post-FC1 or post-FC2 session while non-session specific response neurons showed altered neural response during entire retrieval sessions (Only excited neurons were included) (Friedman test, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001) (Fig. 27D).

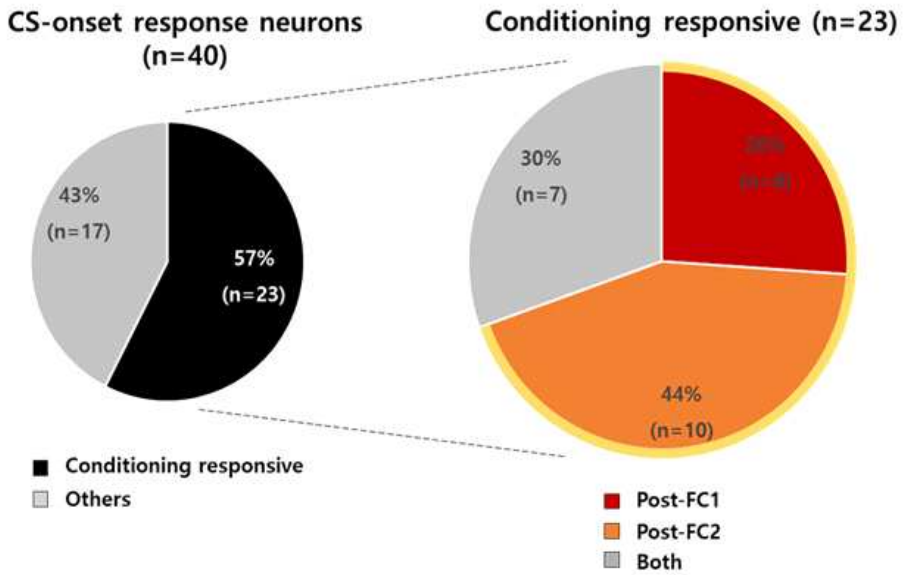


Figure 24. Existence of discerned neural population during repeated retrieval after single fear conditioning with 1day interval.

CS-onset response neurons were found including pre-FC session. Among CS-onset response neurons, 57% (n=23) displayed neural activity alteration as a result of fear conditioning. Within conditioning responsive neurons, 70% (n=16) showed retrieval session-specific response while 30% (n=7) exhibited neural response constantly. Yellow boundary indicates retrieval session-specific response neurons.

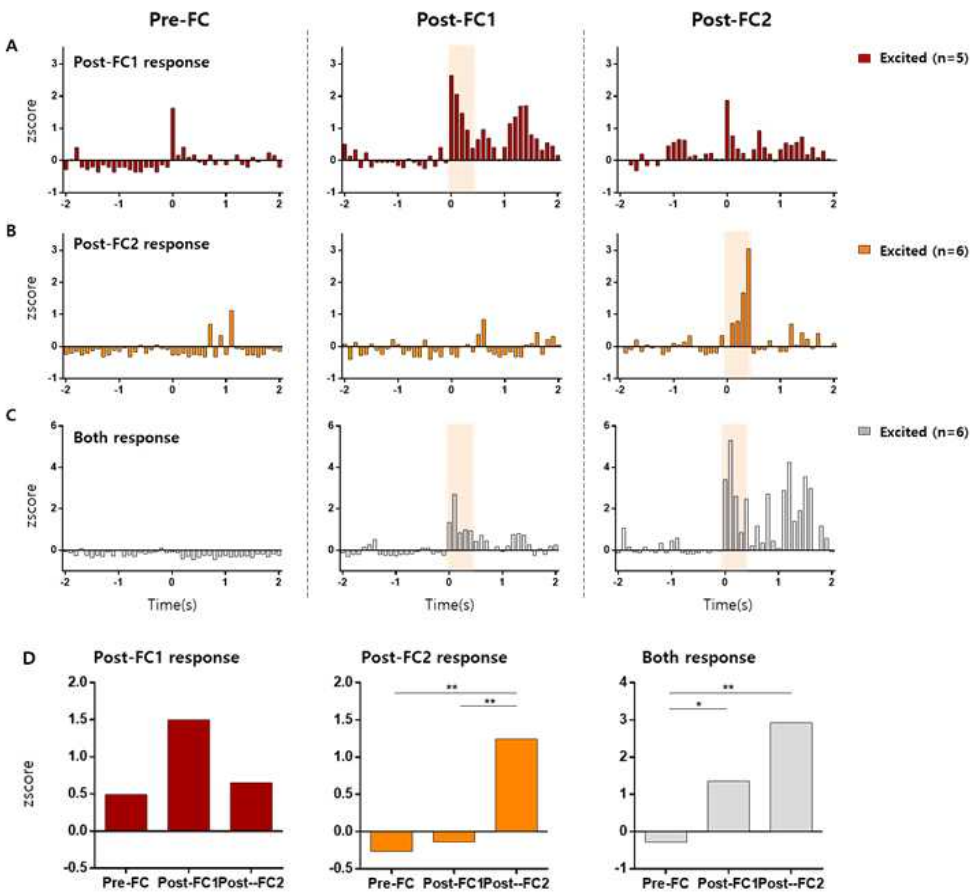

Pre-FC
Post-FC1
Post-FC2
A
Post-FC1 response
B
Post-FC2 response
C
Both response
zscore
Time(s)
Excited (n=5)
Excited (n=6)
Excited (n=6)
D
Post-FC1 response
Post-FC2 response
Both response
Pre-FC
Post-FC1
Post--FC2

Figure 25. BA neural activity during repeated retrieval after fear conditioning with 1day interval.

A, B. Z-score for retrieval session specific CS-onset response neurons. A showed z-score for post-FC1 response neurons and B represented z-score for post-FC2 response neurons. **C.** Z-score for both retrieval session response neurons. They exhibited sustained neural activity during repeated retrieval sessions. **D.** Mean z-score of excited neurons, which showed retrieval session specific or both retrieval session response neurons, while two consecutive retrievals conducted with 1day interval. Retrieval session-specific response neurons presented an increased neural response to post-FC1 or post-FC2 session. Both session response neurons maintained excited neural response during the entire retrieval session. $*p<0.05$, $**p<0.01$.

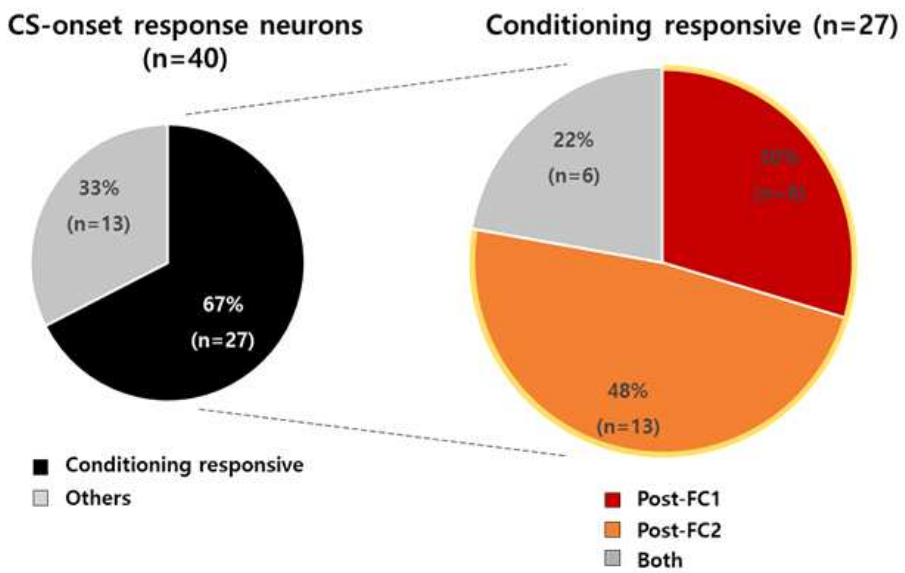


Figure 26. Existence of discerned neural population during repeated retrieval after single fear conditioning with 1hour interval.

CS-onset response neurons were found including pre-FC session. 67% (n=27) of CS-onset response neurons showed CS-onset response followed by fear conditioning. Within conditioning responsive neurons, 78% (n=21) exhibited retrieval session-specific response while 22% (n=6) maintained neural response during repeated retrieval session. Yellow border indicates retrieval session-specific response neurons.

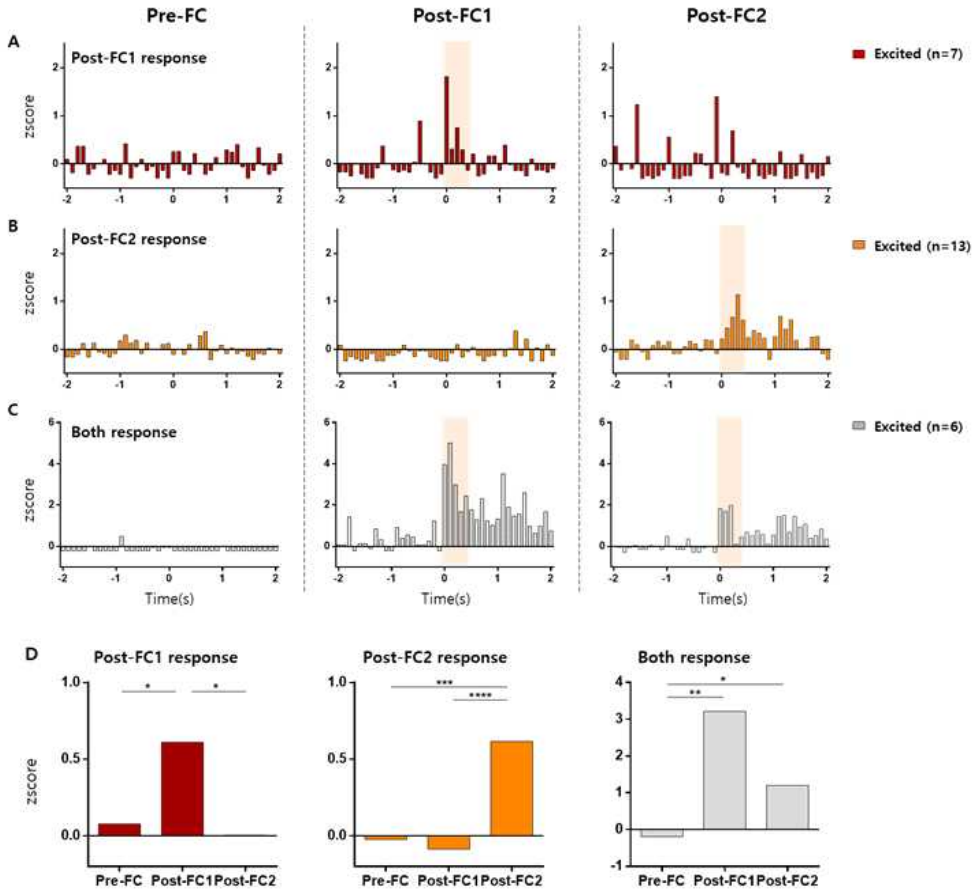

Pre-FC
Post-FC1
Post-FC2
A
Post-FC1 response
zscore
Excited (n=7)
B
Post-FC2 response
zscore
Excited (n=13)
C
Both response
zscore
Excited (n=6)
Time(s)
Time(s)
Time(s)
D
Post-FC1 response
Post-FC2 response
Both response
zscore
Pre-FC Post-FC1 Post-FC2
Pre-FC Post-FC1 Post-FC2
Pre-FC Post-FC1 Post-FC2

Figure 27. BA neural response during repeated retrieval after fear conditioning with 1hour interval.

A, B. Z-score for retrieval session specific CS-onset response neurons. A displayed z-score for post-FC1 response neurons while B showed z-score for post-FC2 response neurons. **C.** Z-score for both post-FC1 and post-FC2 response neurons. Those neurons maintained neural response during repeated retrieval sessions. **D.** Mean z-score of retrieval session specific or both retrieval session response excited neurons during two consecutive retrieval session with 1hour interval. Retrieval session-specific response neurons showed altered neural response to post-FC1 or post-FC2 session. Both session response neurons exhibited excited response to both post-FC1 and post-FC2 sessions. *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

LA neurons exhibited stable neural response during repeated retrieval after fear conditioning with 1hour interval

To confirm whether the LA and the BA have a different role during emotional memory process, I observed neural activity within LA. LA neurons were recorded from the part of the animals, which conducted repeated retrieval after single fear conditioning with 1hour interval (n=6; n=5 included in BA neural analysis). LA neurons identified by histological verification. Behavior results indicated the freezing response altered after the fear conditioning (Fig. 28).

During two consecutive retrieval sessions, 34 neurons were identified and 35% (n=12) neurons showed the CS-onset response during the entire behavior sessions. Followed by the criteria for conditioning dependent response neurons, 67% (n=8) exhibited conditioning-dependent CS-onset response. Interestingly, half of the neurons (n=4) showed neural activity modification at the first retrieval session whereas the last half of the neurons (n=4) displayed neural response alteration at both first and second retrieval sessions. Post-FC2 specific response neurons did not exist (Fig. 29).

Z-score of the post-FC1 response neurons displayed neural response modification during first retrieval sessions (Fig. 30A). On the other hand, both session response neurons maintained neural response modification during the two consecutive retrieval sessions

(Fig. 30B). Mean z-score indicated that post-FC1 response neurons showed a tendency of excited neural response during the post-FC1 session while both response neurons exhibited neural response alteration during both post-FC1 and post-FC2 sessions (Only excited neurons were included) (Fig. 30C).

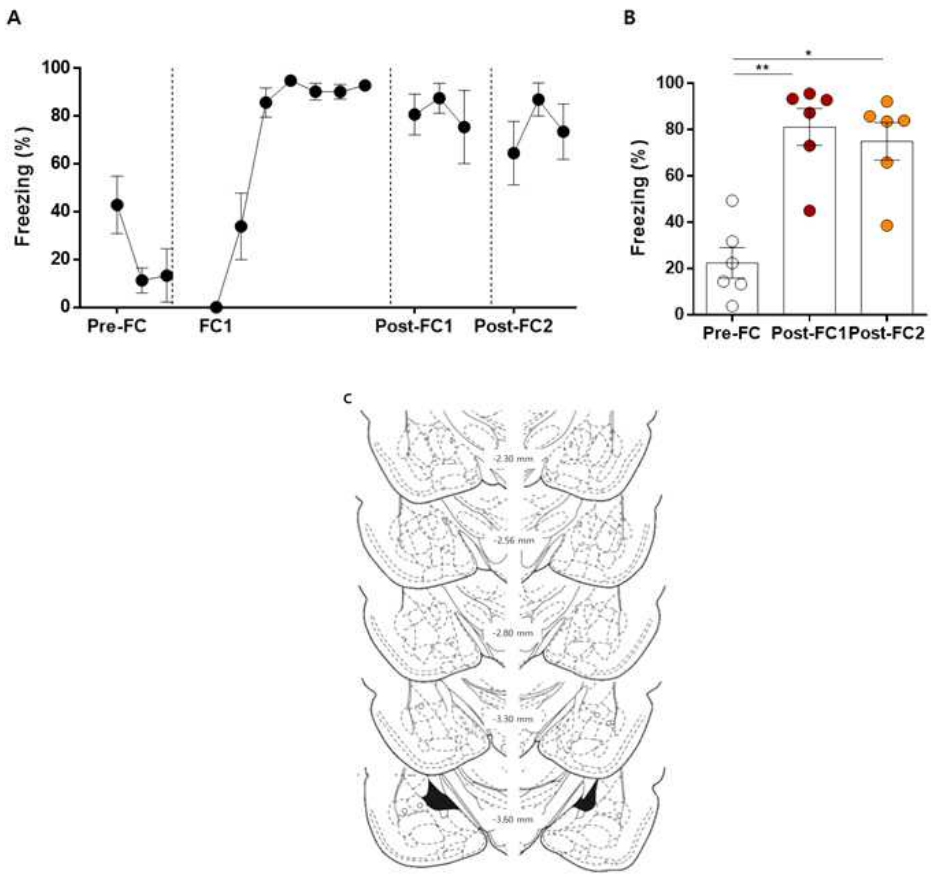

A
Freezing (%)
100
80
60
40
20
0
Pre-FC
FC1
Post-FC1
Post-FC2
B
Freezing (%)
**
*
Pre-FC
Post-FC1
Post-FC2
C
-2.30 mm
-2.56 mm
-2.80 mm
-3.30 mm
-3.60 mm

Figure 28. Freezing behavior during repeated retrieval with 1hour interval and histological verification for LA neural analysis.

A. The learning curve of the entire behavior sessions for the control group (n=6). **B.** Mean freezing response during entire behavior sessions. *$p<0.05$, **$p<0.01$. **C.** Histological verification of electrode placement (n=6). Control groups composed of rats with electrodes placed on the LA.

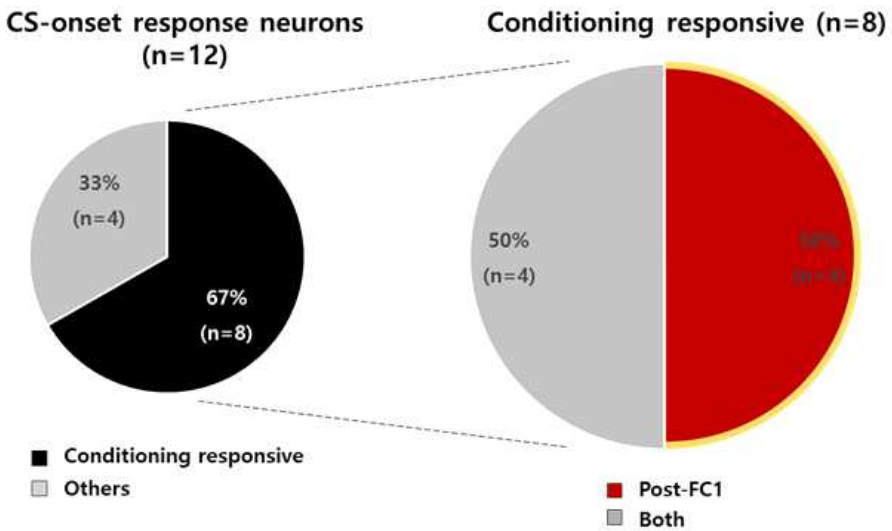


Figure 29. Stable response appeared within the LA during repeated retrieval with 1hour interval.

CS-onset response neurons were found including pre-FC session. 67% (n=8) of CS-onset response neurons displayed neural activity alteration as a result of fear conditioning. Within conditioning responsive neurons, 50% (n=4) showed neural response at first retrieval session while 50% (n=4) maintained neural response continuously. Yellow boundary indicates retrieval session-specific response neurons.

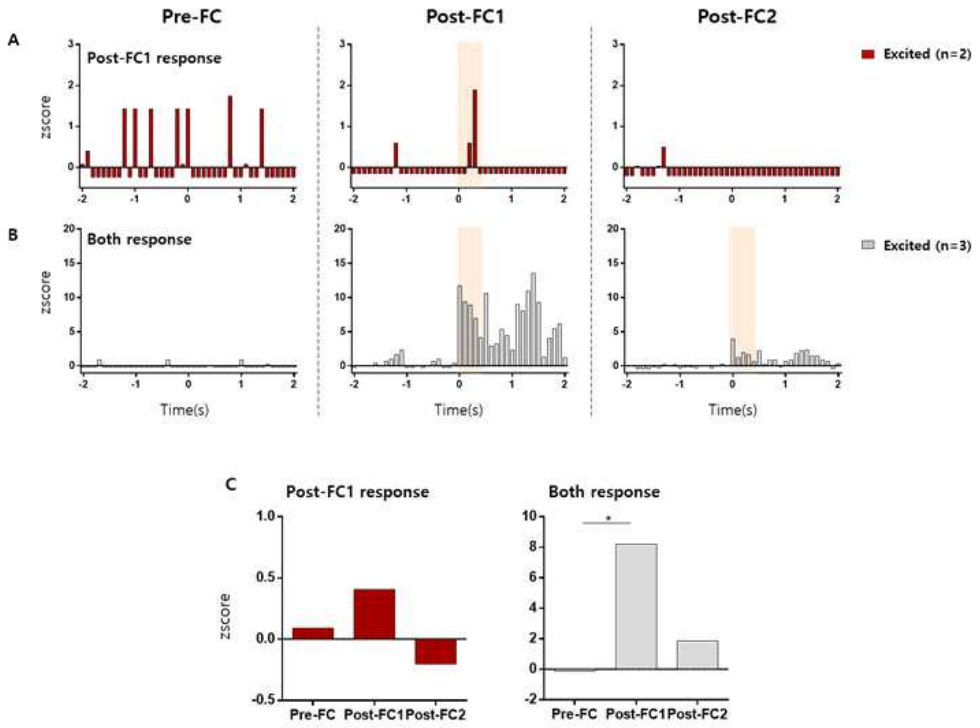


Figure 30. LA neural response during repeated retrieval with 1hr interval.

A. Z-score for post-FC1 specific response neurons during entire behavior sessions. **B.** Z-score for both retrieval session response neurons during entire behavior sessions. **C.** Mean z-score for post-FC1 specific response neurons and both retrieval session-specific response neurons. Excited response neurons included only. $*p<0.05$.

Discussion

In this study, I try to figure out the role of dynamic neural activity within the BA whether they encode emotion-involved event. By doing so, I observed the neural activity throughout the repeated fear conditioning, which composed of a different event with the same value. As a result, 86% of conditioning dependent cue-onset response neurons were classified event-specific response neurons, which showed distinct neural population activity after the same fear conditioning event (Fig. 19). Experimental data from repeated fear conditioning indicate that the distinct population appears continuously followed by the same emotional learning. Therefore, I assume that the dynamic neural activity within the BA encodes emotion-involved event, including value.

Moreover, to prove neural activity appeared during repeated fear conditioning was caused by fear conditioning event, I conducted multiple retrievals after single fear conditioning. Interestingly, I found out that the discerned neural population also appear within each retrieval session even though the proportion of distinct neural population slightly diminished compared to the results from repeated fear conditioning (Fig. 24 and 26). Based on the appearance of the distinct neural population after repeated fear conditioning and multiple retrievals after single fear conditioning, I suggest that the BA is crucial to represent the confronted emotional event, including the

value of the event.

From previous studies, a large body of evidence suggests that the BLA encode valence information by using segregated neural population (Beyeler et al., 2016; Kyriazi et al., 2018). Moreover, some of the other studies indicate the emergence of the distinct neural population followed by cue-dependent value change (Belova et al., 2007; Morrison and Salzman, 2010; Sangha, 2015; Zhang and Li, 2018). I also found the appearance of a different neural population when cue-dependent value updated in chapter 1. However, because the valence encoding process is examined by positive and negative associative learning, which also cause the distinct neural activity within the BLA (Herry et al., 2008; Lee et al., 2017; Quirk et al., 1995; Tye et al., 2010; Tye et al., 2008), the role of the dynamic neural activity during emotional process is unclear. Therefore, the experimental results from repeated fear conditioning and consecutive fear retrieval help to understand the role of distinct neural activity within the BA.

The BLA consist of two different sub-region: LA and BA. The LA is known as a place where CS-US association occur during cue-dependent fear conditioning. On the other hand, the BA is regarded to have a different role compared to the LA, because of their reciprocal connection with other brain regions. Especially, the BA has a connection with other cortical areas, which support that the BA modulates other brain regions based on the emotional information

(Schoenbaum et al., 1998; Sotres-Bayon and Quirk, 2010). However, the majority of previous studies focus on the BLA during emotional behavior process or cue-dependent value updating even though the LA and the BA have a different role during fear conditioning process (An et al., 2012; Han et al., 2009; Reijmers et al., 2007). Therefore, I focus on the neural activity within the BA, which already exhibits dynamic neural activity in chapter 1.

Here, I observed neural activity within the BA and attempted to figure out the role of the BA during the emotional process. To make sure the BA has a different role compared to the LA, I also analyze neural activity within the LA. In the BA, I found out the constant emergence of the different neural population, followed by CS-dependent emotional event (Fig. 19, 24, and 26). However, LA showed a more stable response compared to the BA (Fig. 29). These results indicate that the dynamic neural response appears within the BA, specifically encoding the emotional event.

Several reasons cause the emergence of the segregated neural population during multiple fear retrieval. First of all, retrieval is realized as a new stimulus to the animal. This is because retrieval makes first memory as labile state and reconsolidation occur followed by retrieval (Dudai, 2012; Nader and Hardt, 2009). Therefore, the new population appears as a result of retrieval. However, the distinct population also exist during multiple retrieval behavior with 1hour interval, which is a middle of the reconsolidation process. Therefore,

the cause of the new population cannot be fully explained by retrieval. Second, time flow can affect BA neural activity modification. This is because the previous study indicated that neural connectivity was altered over time (Do-Monte et al., 2015). Therefore, BA neural activity keeps changing as time course. However, 1day is not enough to exclude the BA during the fear retrieval process, so time flow cannot be a source of the distinct neural activity.

As a result, experimental data demonstrate that dynamic neural response appears during emotional memory process within the BA. Furthermore, distinct population activity is continuously emerged, followed by the same emotional event and repeated retrieval about a single event. Together, I suggest that the BA encodes emotional state via distinct neural population.

References

An B, Hong I, Choi S (2012), Long-term neural correlates of reversible fear learning in the lateral amygdala. J Neurosci 32:16845-16856.

Balleine BW, Killcross S (2006), Parallel incentive processing: an integrated view of amygdala function. Trends Neurosci 29:272-279.

Baxter MG, Murray EA (2002), The amygdala and reward. Nat Rev Neurosci 3:563-573.

Bechara A, Damasio AR, Damasio H, Anderson SW (1994), Insensitivity to future consequences following damage to human prefrontal cortex. Cognition 50:7-15.

Belova MA, Paton JJ, Morrison SE, Salzman CD (2007), Expectation modulates neural responses to pleasant and aversive stimuli in primate amygdala. Neuron 55:970-984.

Berridge KC, Robinson TE (2003), Parsing reward. Trends Neurosci 26:507-513.

Beyeler A, Chang CJ, Silvestre M, Leveque C, Namburi P, Wildes CP, Tye KM (2018), Organization of Valence-Encoding and Projection-Defined Neurons in the Basolateral Amygdala. Cell Rep 22:905-918.

Beyeler A, Namburi P, Glober GF, Simonnet C, Calhoon GG, Conyers GF, Luck R, Wildes CP, et al. (2016), Divergent Routing of Positive and Negative Information from the Amygdala during Memory Retrieval. Neuron 90:348-361.

Blair HT, Schafe GE, Bauer EP, Rodrigues SM, LeDoux JE (2001), Synaptic plasticity in the lateral amygdala: a cellular hypothesis of fear conditioning. Learn Mem 8:229-242.

Burgos-Robles A, Vidal-Gonzalez I, Quirk GJ (2009), Sustained conditioned responses in prelimbic prefrontal neurons are correlated with fear expression and extinction failure. J Neurosci 29:8474-8482.

Cai DJ, Aharoni D, Shuman T, Shobe J, Biane J, Song W, Wei B, Veshkini M, et al. (2016), A shared neural ensemble links distinct contextual memories encoded close in time. Nature 534:115-118.

Ciocchi S, Herry C, Grenier F, Wolff SB, Letzkus JJ, Vlachos I, Ehrlich I, Sprengel R, et al. (2010), Encoding of conditioned fear in central amygdala inhibitory circuits. Nature 468:277-282.

Corcoran KA, Quirk GJ (2007), Activity in prelimbic cortex is necessary for the expression of learned, but not innate, fears. J Neurosci 27:840-844.

Do-Monte FH, Quinones-Laracuente K, Quirk GJ (2015), A temporal shift in the circuits mediating retrieval of fear memory. Nature 519:460-463.

Do Monte FH, Quirk GJ, Li B, Penzo MA (2016), Retrieving fear memories, as time goes by. Mol Psychiatry 21:1027-1036.

Dudai Y (2012), The restless engram: consolidations never end. Annu Rev Neurosci 35:227-247.

Felix-Ortiz AC, Beyeler A, Seo C, Leppla CA, Wildes CP, Tye KM (2013), BLA to vHPC inputs modulate anxiety-related behaviors. Neuron 79:658-664.

Felsenberg J, Jacob PF, Walker T, Barnstedt O, Edmondson-Stait AJ, Pleijzier MW, Otto N, Schlegel P, et al. (2018), Integration of Parallel Opposing Memories Underlies Memory Extinction. Cell 175:709-722 e715.

Gallagher M, Holland PC (1994), The amygdala complex: multiple roles in associative learning and attention. Proc Natl Acad Sci U S A 91:11771-11776.

Goosens KA, Hobin JA, Maren S (2003), Auditory-evoked spike firing

in the lateral amygdala and Pavlovian fear conditioning: mnemonic code or fear bias? Neuron 40:1013-1022.

Gore F, Schwartz EC, Brangers BC, Aladi S, Stujenske JM, Likhtik E, Russo MJ, Gordon JA, et al. (2015), Neural Representations of Unconditioned Stimuli in Basolateral Amygdala Mediate Innate and Learned Responses. Cell 162:134-145.

Grewe BF, Grundemann J, Kitch LJ, Lecoq JA, Parker JG, Marshall JD, Larkin MC, Jercog PE, et al. (2017), Neural ensemble dynamics underlying a long-term associative memory. Nature 543:670-675.

Grundemann J, Luthi A (2015), Ensemble coding in amygdala circuits for associative learning. Curr Opin Neurobiol 35:200-206.

Han JH, Kushner SA, Yiu AP, Cole CJ, Matynia A, Brown RA, Neve RL, Guzowski JF, et al. (2007), Neuronal competition and selection during memory formation. Science 316:457-460.

Han JH, Kushner SA, Yiu AP, Hsiang HL, Buch T, Waisman A, Bontempi B, Neve RL, et al. (2009), Selective erasure of a fear memory. Science 323:1492-1496.

Hatfield T, Han JS, Conley M, Gallagher M, Holland P (1996), Neurotoxic lesions of basolateral, but not central, amygdala interfere with Pavlovian second-order conditioning and reinforcer devaluation

effects. J Neurosci 16:5256-5265.

Herry C, Ciocchi S, Senn V, Demmou L, Muller C, Luthi A (2008), Switching on and off fear by distinct neuronal circuits. Nature 454:600-606.

Herry C, Johansen JP (2014), Encoding of fear learning and memory in distributed neuronal circuits. Nat Neurosci 17:1644-1654.

Hiroi N, White NM (1991), The lateral nucleus of the amygdala mediates expression of the amphetamine-produced conditioned place preference. J Neurosci 11:2107-2116.

Holland PC, Gallagher M (2004), Amygdala-frontal interactions and reward expectancy. Curr Opin Neurobiol 14:148-155.

Hong I, Song B, Lee S, Kim J, Kim J, Choi S (2009), Extinction of cued fear memory involves a distinct form of depotentiation at cortical input synapses onto the lateral amygdala. Eur J Neurosci 30:2089-2099.

Hu H (2016), Reward and Aversion. Annu Rev Neurosci 39:297-324.

Janak PH, Tye KM (2015), From circuits to behaviour in the amygdala. Nature 517:284-292.

Johansen JP, Diaz-Mataix L, Hamanaka H, Ozawa T, Ycu E, Koivumaa J, Kumar A, Hou M, et al. (2014), Hebbian and neuromodulatory mechanisms interact to trigger associative memory formation. Proc Natl Acad Sci U S A 111:E5584-5592.

Johansen JP, Hamanaka H, Monfils MH, Behnia R, Deisseroth K, Blair HT, LeDoux JE (2010), Optical activation of lateral amygdala pyramidal cells instructs associative fear learning. Proc Natl Acad Sci U S A 107:12692-12697.

Josselyn SA, Kohler S, Frankland PW (2015), Finding the engram. Nat Rev Neurosci 16:521-534.

Kim J, Lee S, Park K, Hong I, Song B, Son G, Park H, Kim WR, et al. (2007), Amygdala depotentiation and fear extinction. Proc Natl Acad Sci U S A 104:20955-20960.

Kim J, Pignatelli M, Xu S, Itohara S, Tonegawa S (2016), Antagonistic negative and positive neurons of the basolateral amygdala. Nat Neurosci 19:1636-1646.

Kim J, Song B, Hong I, Kim J, Lee J, Park S, Eom JY, Lee CJ, et al. (2010), Reactivation of fear memory renders consolidated amygdala synapses labile. J Neurosci 30:9631-9640.

Kyriazi P, Headley DB, Pare D (2018), Multi-dimensional Coding by Basolateral Amygdala Neurons. Neuron 99:1315-1328 e1315.

LeDoux JE (2000), Emotion circuits in the brain. Annu Rev Neurosci 23:155-184.

Lee SC, Amir A, Haufler D, Pare D (2017), Differential Recruitment of Competing Valence-Related Amygdala Networks during Anxiety. Neuron 96:81-88 e85.

Liu X, Ramirez S, Pang PT, Puryear CB, Govindarajan A, Deisseroth K, Tonegawa S (2012), Optogenetic stimulation of a hippocampal engram activates fear memory recall. Nature 484:381-385.

Livneh U, Paz R (2012), Aversive-bias and stage-selectivity in neurons of the primate amygdala during acquisition, extinction, and overnight retention. J Neurosci 32:8598-8610.

Marek R, Strobel C, Bredy TW, Sah P (2013), The amygdala and medial prefrontal cortex: partners in the fear circuit. J Physiol 591:2381-2391.

Maren S, Fanselow MS (1996), The amygdala and fear conditioning: has the nut been cracked? Neuron 16:237-240.

Maren S, Quirk GJ (2004), Neuronal signalling of fear memory. Nat Rev Neurosci 5:844-852.
McDonald AJ (1998), Cortical pathways to the mammalian amygdala. Prog Neurobiol 55:257-332.

McKernan MG, Shinnick-Gallagher P (1997), Fear conditioning induces a lasting potentiation of synaptic currents in vitro. Nature 390:607-611.

Milad MR, Quirk GJ (2002), Neurons in medial prefrontal cortex signal memory for fear extinction. Nature 420:70-74.

Morrison SE, Salzman CD (2010), Re-valuing the amygdala. Curr Opin Neurobiol 20:221-230.

Murray EA (2007), The amygdala, reward and emotion. Trends Cogn Sci 11:489-497.

Nader K, Hardt O (2009), A single standard for memory: the case for reconsolidation. Nat Rev Neurosci 10:224-234.

Nader K, Majidishad P, Amorapanth P, LeDoux JE (2001), Damage to the lateral and central, but not other, amygdaloid nuclei prevents the acquisition of auditory fear conditioning. Learn Mem 8:156-163.

Namburi P, Beyeler A, Yorozu S, Calhoon GG, Halbert SA, Wichmann R, Holden SS, Mertens KL, et al. (2015), A circuit mechanism for differentiating positive and negative associations. Nature 520:675-678.

O'Neill PK, Gore F, Salzman CD (2018), Basolateral amygdala circuitry in positive and negative valence. Curr Opin Neurobiol 49:175-183.

Pare D, Quirk GJ, Ledoux JE (2004), New vistas on amygdala networks in conditioned fear. J Neurophysiol 92:1-9.

Paton JJ, Belova MA, Morrison SE, Salzman CD (2006), The primate amygdala represents the positive and negative value of visual stimuli during learning. Nature 439:865-870.

Phelps EA, LeDoux JE (2005), Contributions of the amygdala to emotion processing: from animal models to human behavior. Neuron 48:175-187.

Phillips RG, LeDoux JE (1992), Differential contribution of amygdala and hippocampus to cued and contextual fear conditioning. Behav Neurosci 106:274-285.

Pickens CL, Saddoris MP, Setlow B, Gallagher M, Holland PC,

Schoenbaum G (2003), Different roles for orbitofrontal cortex and basolateral amygdala in a reinforcer devaluation task. J Neurosci 23:11078-11084.

Quirk GJ, Armony JL, LeDoux JE (1997), Fear conditioning enhances different temporal components of tone-evoked spike trains in auditory cortex and lateral amygdala. Neuron 19:613-624.

Quirk GJ, Repa C, LeDoux JE (1995), Fear conditioning enhances short-latency auditory responses of lateral amygdala neurons: parallel recordings in the freely behaving rat. Neuron 15:1029-1039.

Rashid AJ, Yan C, Mercaldo V, Hsiang HL, Park S, Cole CJ, De Cristofaro A, Yu J, et al. (2016), Competition between engrams influences fear memory formation and recall. Science 353:383-387.

Redondo RL, Kim J, Arons AL, Ramirez S, Liu X, Tonegawa S (2014), Bidirectional switch of the valence associated with a hippocampal contextual memory engram. Nature 513:426-430.

Reijmers LG, Perkins BL, Matsuo N, Mayford M (2007), Localization of a stable neural correlate of associative memory. Science 317:1230-1233.

Repa JC, Muller J, Apergis J, Desrochers TM, Zhou Y, LeDoux JE

(2001), Two different lateral amygdala cell populations contribute to the initiation and storage of memory. Nat Neurosci 4:724-731.

Rich MT, Huang YH, Torregrossa MM (2019), Plasticity at Thalamo-amygdala Synapses Regulates Cocaine-Cue Memory Formation and Extinction. Cell Rep 26:1010-1020 e1015.

Rogan MT, Staubli UV, LeDoux JE (1997), Fear conditioning induces associative long-term potentiation in the amygdala. Nature 390:604-607.

Rozeske RR, Jercog D, Karalis N, Chaudun F, Khoder S, Girard D, Winke N, Herry C (2018), Prefrontal-Periaqueductal Gray-Projecting Neurons Mediate Context Fear Discrimination. Neuron 97:898-910 e896.

Russell JA (1980), A Circumplex Model of Affect. Journal of Personality and Social Psychology 39:1161-1178.

Salzman CD, Fusi S (2010), Emotion, cognition, and mental state representation in amygdala and prefrontal cortex. Annu Rev Neurosci 33:173-202.

Sangha S (2015), Plasticity of Fear and Safety Neurons of the Amygdala in Response to Fear Extinction. Front Behav Neurosci

9:354.

Sangha S, Chadick JZ, Janak PH (2013), Safety encoding in the basal amygdala. J Neurosci 33:3744-3751.

Schoenbaum G, Chiba AA, Gallagher M (1998), Orbitofrontal cortex and basolateral amygdala encode expected outcomes during learning. Nat Neurosci 1:155-159.

Schultz W (2000), Multiple reward signals in the brain. Nat Rev Neurosci 1:199-207.

Sengupta A, Yau JOY, Jean-Richard-Dit-Bressel P, Liu Y, Millan EZ, Power JM, McNally GP (2018), Basolateral Amygdala Neurons Maintain Aversive Emotional Salience. J Neurosci 38:3001-3012.

Shabel SJ, Janak PH (2009), Substantial similarity in amygdala neuronal activity during conditioned appetitive and aversive emotional arousal. Proc Natl Acad Sci U S A 106:15031-15036.

Sierra-Mercado D, Padilla-Coreano N, Quirk GJ (2011), Dissociable roles of prelimbic and infralimbic cortices, ventral hippocampus, and basolateral amygdala in the expression and extinction of conditioned fear. Neuropsychopharmacology 36:529-538.

Sotres-Bayon F, Quirk GJ (2010), Prefrontal control of fear: more than just extinction. Curr Opin Neurobiol 20:231-235.

Stalnaker TA, Franz TM, Singh T, Schoenbaum G (2007), Basolateral amygdala lesions abolish orbitofrontal-dependent reversal impairments. Neuron 54:51-58.

Stuber GD, Sparta DR, Stamatakis AM, van Leeuwen WA, Hardjoprajitno JE, Cho S, Tye KM, Kempadoo KA, et al. (2011), Excitatory transmission from the amygdala to nucleus accumbens facilitates reward seeking. Nature 475:377-380.

Tovote P, Fadok JP, Luthi A (2015), Neuronal circuits for fear and anxiety. Nat Rev Neurosci 16:317-331.

Tseng WT, Yen CT, Tsai ML (2011), A bundled microwire array for long-term chronic single-unit recording in deep brain regions of behaving rats. J Neurosci Methods 201:368-376.

Tye KM, Cone JJ, Schairer WW, Janak PH (2010), Amygdala neural encoding of the absence of reward during extinction. J Neurosci 30:116-125.

Tye KM, Stuber GD, de Ridder B, Bonci A, Janak PH (2008), Rapid strengthening of thalamo-amygdala synapses mediates cue-reward learning. Nature 453:1253-1257.

Wolff SB, Grundemann J, Tovote P, Krabbe S, Jacobson GA, Muller C, Herry C, Ehrlich I, et al. (2014), Amygdala interneuron subtypes control fear learning through disinhibition. Nature 509:453-458.

Yiu AP, Mercaldo V, Yan C, Richards B, Rashid AJ, Hsiang HL, Pressey J, Mahadevan V, et al. (2014), Neurons are recruited to a memory trace based on relative neuronal excitability immediately before training. Neuron 83:722-735.

Zhang X, Li B (2018), Population coding of valence in the basolateral amygdala. Nat Commun 9:5195.

국문초록

편도체의 감정 가치 정보 생성기전

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편도체는 감정정보를 처리하는데 중요하게 작용하는 뇌 부위로 감정정보 중 특히 공포기억에 대한 연구가 신경생리학적인 방법을 통해 많이 연구되었다. 또한 편도체는 공포기억 외에도 보상 기억, 걱정, 불안, 사회적 상호작용과 같은 다양한 감정 정보 과정에 관여한다고 알려져 있다.

감정은 크게 두 가지 요소인 가치 정보와 각성 정보로 구분되어

지며, 감정 정보를 처리하는데 있어 편도체 내에서 두 가지 정보가 모두 처리된다고 알려져 있다. 특히 편도체에서 특정 뇌 부위로 가는 신경세포 집단이 존재하며, 가치 정보는 서로 다른 자극에 대해 특정 뇌 부위로 가는 신경세포 집단의 일부에 의해 처리되는 반면 각성 정보의 경우 동일한 신경세포 그룹이 다양한 자극들에 대해 반응하는 것으로 알려져 있다.

그러나 감정 정보를 처리하는데 있어 신경세포의 활성을 살펴본 대다수의 논문들은 특정 시점에서의 신경세포의 활성을 관측했기 때문에 시간에 흐름에 따른 편도체 신경세포 활성 변화를 관측하지 못했으며 이로 인해 사건의 흐름에 따라 편도체 신경세포의 활성이 어떻게 변화하는지 살펴볼 수 없었다.

이번 연구에서는 단일 신경세포의 활성을 장기간 살펴봄으로써 기초 편도체의 역할을 알아보고자 하였다.

제 1장에서는 먼저 기초 편도체가 이전 연구결과들과 마찬가지로 보상 조건화 학습과 공포 조건화 학습을 진행하였을 때 서로 다른 신경세포의 활성을 보이는지 확인하였다. 나아가 각 보상 기억과 공포 기억의 소멸 과정에서 새로운 신경세포들의 활성이 나타나는지 관측하였다. 결과 각 보상 기억과 공포 기억의 소멸과정에서 새로운 신경세포들이 활성화되는 것을 확인하였으며, 이는 공포 조건화 학습 및 보상 조건화 학습에서 활성화되었던 그룹과도 차이를 보이는 것을 확인하였다. 감정 기억의 소멸 과정에 새로운 신경세포들이 활성화되는 결과를 통해 감정 기억의 소멸 과정은 새로운 가치 정보를 필요로 함을 확인할 수 있었다.

다만 이러한 특이적인 신경세포 그룹의 활성은 각 세션에 반응

성을 보이는 것이기 때문에 기초 편도체가 감정 정보에서 나아가 감정 사건을 처리할 가능성이 있었다.

이를 시험하기 위해 제 2장에서는 공포 조건화 학습을 반복적으로 진행하는 동안 기초 편도체의 단일 세포 활성을 관찰하였다. 결과 반복적인 공포 조건화 학습 역시 새로운 신경세포 그룹의 활성을 유도하는 것을 확인할 수 있었다. 나아가 공포 조건화 학습 후 기억 인출을 반복적으로 진행하였을 경우에도 새로운 신경세포 그룹의 활성이 나타나는 것을 확인하였다. 이러한 결과들을 바탕으로 기초 편도체가 감정적 사건을 처리하는데 관여하는 것을 확인할 수 있었다.

핵심어: 기초편도체, 가치, 각성, 공포 조건화 학습, 보상 조건화 학습

학번: 2013-23004