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Reward expectation alters learning and memory: The impact of the amygdala on appetitive-driven behaviors

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Abstract

The capacity to seek and obtain rewards is essential for survival. Pavlovian conditioning is one mechanism by which organisms develop predictions about rewards and such anticipatory or expectancy states enable successful behavioral adaptations to environmental demands. Reward expectancies have both affective/motivational and discriminative properties that allow for the modulation of instrumental goal-directed behavior. Recent data provides evidence that different cognitive strategies (cue-outcome associations) and neural systems (amygdala) are used when subjects are trained under conditions that allow Pavlovian-induced reward expectancies to guide instrumental behavioral choices. Furthermore, it has been demonstrated that impairments typically observed in a number of brain-damaged models are alleviated or eliminated by embedding unique reward expectancies into learning/memory tasks. These results suggest that Pavlovian-induced reward expectancies can change both behavioral and brain processes.

Keywords

reward; amygdala; Pavlovian conditioning; Instrumental learning; differential outcomes

1. Pavlovian associations produce specific reward expectancies that can modulate instrumental behavior

Expectations of reward can have powerful effects on learning and performance. Learning to anticipate a reward can occur by associating a discrete stimulus (S-O; Pavlovian conditioning) and/or an action (R-O; instrumental conditioning) with an outcome (see Figure 1-A). A predictive cue such as a conditioned stimulus (CS) or discriminative stimulus (S^D) can enter into associations with either the general motivational properties of the reward or the sensory specific properties of reward [see 23; 41]. It has been demonstrated that reward expectancies produced by Pavlovian procedures can therefore have affective/motivational and sensory/discriminative properties that mediate instrumental behavior [for reviews see: 94; 116]. Understanding the interactions between Pavlovian-Instrumental behaviors was originally investigated under the two-process theory [64, 65]. In many natural and experimental contexts

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there exists cues (S-stimulus) that predict reward (O-outcome) and goal-directed behavior that is learned (R-response) to gain access to the reward. Mower stated that these cues can function as CS and come to elicit conditioned responses—related to the outcome—that then can motivate instrumental or goal directed behavior. Thus, two-process theory evoked a Pavlovian conditioned anticipatory state (later termed expectancy) as a mediator between the stimulus and the instrumental response (71 -see Figure 1-B).

The Pavlovian-Instrumental interaction involves a three-term contingency: a stimulus (S), a response (R) and an outcome (O). A number of binary associations can be formed: S-O, S-R, R-O and even backwards O-R [23;116]. Through a procedure called Pavlovian-Instrumental transfer (PIT), it has been established that Pavlovian trained conditioned stimuli (CS's) under certain conditions can facilitate instrumental actions [28;70;112;87]. For example, when a specific R (lever press) is trained to be made in the presence of a specific stimulus (S^1 = tone) by positive reinforcement (*Instrumental training phase: S-R*) with a given outcome (O^1 =food pellet), after which, in another phase, a CS (S^2 =clicker) paired with that same outcome (*Pavlovian pairing phase: S^2 - O^1*) and later when this CS (S^2) is imposed into an instrumental paradigm (*Transfer phase*) it can often enhance responding for that specific outcome [112]. Thus, a stimulus (S^2) that has never been associated with the response, acquires the capacity to evoke that response, presumably through overlapping associations with the outcome—both stimuli (tone, clicker) share a common specific reward expectation. The predictive relationship between the S and the O, in this case food, endows the S motivational properties--conditioned incentive motivation. Thus, there is no disputing the data that Pavlovian procedures can imbue a S with the capacity to modulate instrumental behavior [112]. However, the question is whether such a S evokes general motivational or sensory specific features of the reward that then modify instrumental behavior [87;112]. Early learning theorists [64;108] stated that the key property of the Pavlovian mediator was a nonspecific-general motivation of behavior. More contemporary two-process theories argue that CSs possess both general motivation influences through direct association with the hedonic aspects of a reward and specific motivational influences through associations with the sensory specific features of the reward [14;23;41]. Questions that have emerged are whether these two reward-associated properties of a CS differentially affect behavior and whether are they neurobiologically distinct.

Two paradigms have been used to answer these questions from both behavioral and neurobiological standpoints: Reinforcer Devaluation and the Differential Outcomes Procedure. First, below we will describe what we have learned about the associative nature of reward expectancies using these paradigms. After which, in *section 4*, we will examine the potential neural correlates of reward expectations

Reinforcer devaluation is a procedure in which a biologically relevant stimulus (unconditioned stimulus: UCS) is devalued by either pairing the UCS with an agent that makes the organism sick (i.e. lithium chloride) or by allowing the animal to consume as much of the UCS as it wants prior to testing (satiation). This manipulation can be assessed with Pavlovian procedures (measuring orientation/approach behaviors) or with instrumental procedures (measuring shaped behaviors such lever press or chain pull). Under either manipulation, if performance is governed by reward expectation than either type of behavior should be altered when the reward status changes and the cue that predicts the reward is presented [43]. Recent research using the devaluation procedure to assess Pavlovian-Instrumental interactions has revealed a number of important points in regards to reward expectancies. First, there are both sensory specific and general motivational influences of reward that can mediate conditioned as well as instrumental responses [14]. However, different experimental conditions could determine whether general or specific reward expectancies modulate choice behavior. A series of experiments by Holland [41] delineated some of the critical parametric issues that elicit the use of general or specific reward expectations. The data obtained by Holland [41] demonstrated that two experimental

conditions (multiple vs single reward exposure and amount of instrumental training) do affect whether the general or specific features of reward expectation modulate behavior. With a single US, devaluation of that reward reduces baseline instrumental responding after minimal or moderate levels of training, but not after extensive training (Exp 1). This occurs despite the fact that as instrumental training is extended, the ability of a CS to augment instrumental responding actually increased. In the devaluation procedure, there is data that suggests that as training on instrumental performance increases there is a transfer of association from outcome expectancy to S-R mechanisms [2]. However, when animals are trained with multiple cues, reinforcers and responses, the use of detailed or specific reward expectancies persist regardless of extended training [41; Exp 2]. Under the multiple S, R, O paradigm, when a specific reward is devalued, selective responding (associated with that O) was disrupted. Thus, variations in experimental parameters can change the type of information (sensory specific or general motivation) that is associated between cues and reward. Furthermore, we will also see that such associative relationships also determine which brain regions are evoked (see further descriptions below) to remember cue and reward information.

Trapold [111] used a unique paradigm to assess whether organisms use specific anticipatory reward information to guide instrumental behavioral choices. He assumed that animals encode many properties of the reinforcer (sensory, perceptual, magnitude, location) and these properties can enter into association with the sample stimuli, choice stimuli and the response requirement. Rather than having two separate phases for Pavlovian and Instrumental conditioning, he combined them into a single training phase. He had rats solve a discrimination problem that required a response to one lever (R1) in the presence of one stimulus (S1= tone), which was reinforced with a pellet (O1), and to produce a different response on a second lever (R2) in the presence of another stimulus (S2=click) that was reinforced with a different reinforcer-sucrose solution (O2). He found that rats trained with these conditions, the Differential Outcomes Procedure (DOP), mastered the task much more rapidly and to a higher asymptote than subjects exposed to a common reinforcer or multiple reinforcers that were not correlated with stimuli or responses (Nondifferential Outcomes Procedure-NOP). Variations of this powerful effect have been termed the Differential Outcomes Effect (DOE). The DOE is quite robust and can be seen across numerous tasks, parameters, and species [see 33;85;94; 116 for reviews].

The DOP has been successfully used to analyze the nature of associative structure when Pavlovian cues influence instrumental behavior [116]. A number of parameters have been manipulated using various conditional discrimination tasks to determine the behavioral mechanisms responsible for the DOE. It has been suggested that the higher accuracy obtained under the DOP, relative to the NOP, reflects greater stimulus control, response-mediated behavior, or a different memory process. Originally, when the DOE was discovered, Trapold & Overmier [112] hypothesized that the subject learns something specific about the sample-reward relationship. They theorized that through the mechanisms of Pavlovian conditioning the subject learned something specific about the reward (reward specific sensory information) and that this “conditioned expectation” of the reward had stimulus-like or cue properties that guided instrumental behavior--rather than merely providing general motivation.

Support for the hypothesis that reward expectancies, produced by Pavlovian associations, control performance on instrumental tasks when the DOP is used came from both PIT (stimulus substitution; see 51;112] and reversal-transfer [switching sample-reward contingencies- see 75;118] experiments. These manipulations have revealed that subjects are extremely sensitive to specific sample-reward relationships. When another CS is paired with the same reward off line of the instrumental task and then is inserted into the discrimination task (PIT), this new CS can easily substitute for the previous discriminative stimulus [see 51]. In addition, after many specific CS-Outcome pairings (i.e., CS¹-Outcome¹: CS²-Outcome²) that lead to

asymptote responding, switching sample-outcome pairings (CS^1 -Outcome²; CS^2 -Outcome¹) can result in behavior falling to chance level [75].

Thus, these studies demonstrate that outcome expectancies do have functional stimulus-like properties and can serve as reliable cues to guide behavior. The unique reward expectancies had both affective/motivational and sensory discriminative properties which appear to mediate choice behavior independent of sample information [see 44;115;117].

However, it is possible that other associations (R-O; S-R) are created using the DOP and may contribute to performance [24;86]. Embedded within the each procedure is also a correlation between a particular choice alternative (R) and its corresponding unique outcome. This relation may result in response-outcome association (R-O), which may mediate enhanced performance. In the DOP, Sherburn & Zentall [105] have demonstrated that these R-O associations play a minor role relative to the S-O association in mediating the DOE. This interpretation was further supported by Urcuioli and collaborators using a modified partial outcome reversal technique to evaluate the impact of S-O and R-O associations on pigeons' delayed matching [118]. They concluded that S-O associations have a greater control than R-O associations over performance when subjects are trained with the DOP [see 116 for a review].

In summary, both the DOP and the Devaluation-PIT procedure demonstrate that there are both general motivation and sensory-specific aspects to reward expectation and experimental conditions dictate which feature gains control over behavior. Furthermore, there is also evidence that different parts of the amygdala control these different aspects of reward [see 19;42]. Thus, when environmental demands evoke unique sensory specific reward expectation, behavioral control can be transferred to a different brain region. This hypothesis has driven our laboratory to investigate using the DOP as a type of remediation after brain damage.

2. Reward expectancies produced by the DOP can change behavioral success after brain damage

Our laboratory [98;94;95;84] proposed that the DOP allows subjects to use reward expectancies to solve the task and this cognitive process activates different brain regions compared to when a subject must rely on retrospective recall of information (as must be done under NOP conditions) to solve a task. The reliance on reward-related processes activates a memory system that is different, structurally and associatively, from the system commonly used to solve conditional discrimination tasks [see 83;84;94;95;96]. This assertion has been supported by our research program that has demonstrated that rats with various brain disturbances (rats with diencephalic lesions, hippocampal lesions and cholinergic lesions, as well as aged rats) that result in significant impairment on certain types of discrimination tasks, display no-- or dramatically reduced-- impairment when trained on these same tasks with the DOP (see Figure 2).

For example, pyriethamine-induced thiamine deficiency (PTD) in the rat has been used to model the neuroanatomical, neurochemical, and behavioral impairments observed in humans with Wernicke-Korsakoff Syndrome (WKS). Rats with PTD-induced lesions are impaired in what has been termed "hippocampal-dependent tasks" such as delayed alternation [56], matching or nonmatching-to-sample or position [52;56] and working memory assessment on the radial arm maze [88].

We implemented the DOP into the T-maze matching-to-position task (MTP) to determine if it would attenuate the acquisition deficits previously reported in PTD-treated rats [52]. Pair-fed [PF] and PTD-treated rats were divided into DOP and NOP conditions and then trained to acquire the MTP task. Reinforcers for correct responses were chocolate milk or mashed Purina

rat chow. For the DOP condition, a specific reinforcer was given for each correct response (e.g., turn left = chocolate milk, turn right = Purina rat chow). For the NOP group, the reinforcer delivered was randomly determined for each response (e.g., turn left = chocolate milk or Purina rat chow, turn right = chocolate milk or Purina rat chow). When the number of trials required to acquire the MTP task was examined, the PTD-treated rats trained under NOP required about twice as many trials to acquire the task than did the PF control rats trained under NOP—and this is what has been seen when a single common reinforcer has been used [97]. However, when trained with the DOP, the PTD-induced deficit was eliminated: PTD-treated rats and control did not differ in the number needed to master the task. Thus, by implementing the DOP, the traditional learning impairments produced by diencephalic lesions on this task were not seen.

Another memory dysfunction model we examined to determine if the DOP would enhance memory performance was aged rats. Both a subset of humans and rats develop memory problems associated with advanced age. Aged rats are impaired on tasks that rely on relational memory for complex spatial stimuli, but perform similarly to young animals on tasks that involve simple associations [5;31]. We embedded the DOP into an operant delayed Matching-To-Position (DMTP) task, a task that aged rats are impaired [98]. We, and others, have proposed that the information that a subject relies on to solve the DMTP task under DOP conditions is distinct reward expectations [94;116]. After several training sessions, a subject learns to predict which outcome will be delivered when a correct choice response is made and develops a distinct expectation of each unique reward event upon presentation of the sample stimulus. We believe that what directs behavior over the delay interval is reward expectation.

Aged and young rats were divided into two groups: Those trained with the DOP and those trained with the NOP. In this task a lever (right or left) is inserted into the chamber as the sample cue, the animal must respond to that lever to make it retract and advance to the next phase. After the rat pressed the sample lever, it makes a nose poke into the center aperture that causes the choice levers to extend. Working memory was assessed by insertion of a delay interval (0–48 s) between the conclusion of the sample phase and the initiation of the choice phase. A correct choice response is to press the lever that was presented during the sample phase. If the rat made a correct “matching” choice response it received a reinforcer. In the DOP condition, correct choice responses to the left lever were rewarded with a 45 mg food pellet and correct responses to the right lever were rewarded with 0.2 ml of sucrose solution. However, in the NOP condition correct responses, regardless of lever, were randomly rewarded with food pellets or sucrose solution.

Aged rats, relative to young rats, trained with the NOP displayed a dramatic delay-dependent impairment of working memory performance -- similar to when a common outcome is used [27]. However, if subjects are trained with the DOP, either young or aged, enhanced memory performance is observed. Most importantly, when the DOP was used there were no significant differences in memory performance between young and aged rats. As shown in Figure 2, aged rats trained with the DOP do not show the traditional age-related decline in working memory performance.

A similar phenomenon was observed in rats with extensive hippocampal lesions [95]. Although the hippocampal-lesioned animals took more trials to learn the MTP task—regardless of DOP or NOP-- all rats were eventually able to reach criteria of 90% or greater for three sessions when memory functions were not tapped to a great degree on the MTP task. However, when transferred to the delayed MTP paradigm, differences as a function of reinforcement condition (DOP, NOP) and lesion status (hippocampus-lesioned, cortical-lesioned control) began to emerge. Hippocampal-lesioned rats trained with the DOP had a mild transient impairment that diminished quickly (2 sessions) as training progressed. In contrast, rats with hippocampal-

lesions trained with the NOP had severe and persistent impairments in memory-related performance as shown in Figure 2.

Furthermore, rats that received intraventricular injections (ICV) of the cholinergic neurotoxin 195-IgG Saporin (which destroys over 85% of cholinergic neurons in the basal forebrain) do not display memory impairment on DMTP when trained with the DOP. However, under NOP conditions this cholinergic lesion produces significant memory impairment that was present for many sessions (see Figure 2).

It is important to note that the DOP does not protect against the amnesic effects of all manipulations. We have demonstrated that when the DOP is implemented, performance on DMTP is more sensitive to the systemic effects of MK-801, but less sensitive to the effects of scopolamine; however, the reverse is true for the performance of rats trained under NOP conditions [99]. Furthermore, ICV administration of muscimol produced a *delay-independent* effect on memory performance in rats trained with the DOP: performance was disrupted at all delay intervals [see Figure 3 and 83]. In contrast, when rats were trained with the NOP, ICV administration of muscimol produced a *delay-dependent* effect of impairing memory performance only at the longer delay intervals (see also Figure 3).

Thus, there are circumstances in which brain alterations can disrupt behavior guided by reward expectancies (DOP) to either a greater degree or result in a different type of cognitive disruption, when contrasted to subjects have to remember sample information (NOP). This is not unexpected as we speculated that different brain regions are recruited when reward expectancies drive behavioral choices: Therefore, damage to those systems should selectively result in behavioral impairment under conditions when reward expectancies are evoked.

There is now evidence that the amygdala and related brain regions are critical for behavior guided by Pavlovian produced outcome expectancies. The next sections will review the structure and function of the amygdala circuit and how different regions modulate outcome information.

3. Functional evidence for the role of the amygdala in Pavlovian associative processes

There is an accumulation of evidence from a number of studies that the amygdala is critical for expectancy of both aversive and appetitive outcomes. In all species examined, the amygdala plays an important role in Pavlovian conditioning and significant progress has been made in understanding the neural mechanisms of Pavlovian-conditioned fear. For example, functional neuroimaging studies indicate an increased hemodynamic response in the human amygdala during presentation of CSs such as faces, previously paired with a loud auditory US [13;17; reviewed in 12]. Not surprisingly, lesions of the amygdala in humans [7] and rodents [48] block responses to CSs previously paired with aversive outcomes—such as an electrical shock. These data point to the critical involvement of the amygdala in expectancy of aversive outcomes.

The amygdala is a heterogenous telencephalic structure made up of several discrete nuclei that are visible with simple histological stains (ex. Nissl stain; See Figure 4). The presence of different nuclei and nuclear subdivisions [up to 13 different subdivisions; reviewed in 78], possessing distinct cell-types and afferent/efferent connections have resulted in varying classification schemes and nomenclature. Several species differences in amygdalar anatomy have also added to the highly debated classification of the amygdala [109]. However, for the purposes of this review, we will focus primarily on known anatomy of physiology of the rodent amygdala, as most studies of Pavlovian conditioning, including work in our own laboratory, have been performed with rodents. In particular, we will focus on subdivisions of the amygdala

with clear effects on Pavlovian-induced expectancies as revealed by pharmacological inactivation and lesions studies. These behaviorally-relevant nuclei include the basolateral nucleus (BLA, that includes basal and lateral nuclei and their respective subdivisions) as well as the central nucleus (CeA).

Outcome expectancies are triggered by sensory stimuli that have been paired with rewarding or aversive stimuli. Therefore, as the neuroanatomical site for the formation of expectancy, it is critical to first understand how sensory signals reach the amygdala. Numerous studies have detailed the pathways of all sensory modalities and nociception into the amygdala. For each of the sensory systems, the primary target in the amygdala is the BLA, though the origin of these inputs varies according to sensory modality including inputs from brainstem nuclei, thalamic nuclei [55;54], neocortical and/or archicortex regions [59;62]. In addition, hippocampal formation afferents to the BLA provide important multimodal “contextual” cues including that related to spatial and temporal information [49]. Moreover, known projections originating from prefrontal cortex to the BLA [62] also likely provide multimodal information to the amygdala. Thus, the BLA is in a key neuroanatomical position to process of conditioned stimuli that signal relevant outcomes.

The BLA appears to be the primary site where outcome expectancies are formed. The convergence of information about neutral stimuli and affective stimuli in the BLA make this nucleus the ideal site for plastic changes necessary for Pavlovian conditioning. Using Pavlov’s classic conditioning paradigm as an example, auditory information encoding the ringing of a bell converges in the BLA with gustatory information encoding the taste of food. Individual BLA neurons receiving both auditory afferents from medial geniculate nucleus thalamus and/or auditory cortex [25;26] as well as gustatory afferents from the parabrachial nucleus [67] would be potentiated by Hebbian-type mechanisms [reviewed in 10;89;107]. Converging inputs to the BLA signaling neutral stimuli (CS) and affective stimuli (US) would have to occur with high contingency and/or contiguity, in order for plasticity to take place - similar to the contingency/contiguity of CSs and USs necessary to produce learning during Pavlovian training. Physiological studies have indeed demonstrated converging inputs from different sensory modalities onto individual BLA neurons. For example, using single-unit recordings *in vivo* in the BLA, [90] observed neurons that responded to both footshock as well as auditory stimuli. Given the robust conditioning (freezing response) that is observed after tone-footshock pairings, these data provide the cellular and synaptic mechanisms by which outcome expectancies are formed in the BLA [reviewed in Maren 58;107].

An increasing number of physiological studies point to the encoding of outcome expectancy in the firing of BLA neurons. For example, using an auditory fear conditioning paradigm, Quirk and colleagues [82;81] demonstrated an increase in CS-evoked firing in BLA neurons as well as an increase in the number of neurons that fired in response to the tone CS. These data indicate increased sensitivity to CSs encoded by the firing rate of BLA neurons following conditioning. More recently, Collins & Paré [18] used a two-tone conditional discrimination task while performing *in vivo* recordings from BLA neurons. These authors demonstrated increased responding of neurons during tone-CS+ presentations after repeated pairing with a footshock. Interestingly, a decrease in firing rate was observed following presentation of the CS- [18]. These data indicate the ability of BLA neurons to signal stimuli that predict an affective US with increases in firing rate. Equally important is the finding that BLA neurons respond in unique patterns to stimuli that signal the absence of USs.

Whereas the BLA can be thought of as input nucleus for outcome expectancy learning, the central nucleus (CeA; and its subdivisions) can be thought of as the motor output nucleus for behavioral expression of outcome expectancies. The CeA (medial subdivision) sends afferents to the hypothalamus, midbrain, and medullary nuclei [45;119;91]. These targets control the

complex and dynamic behaviors often observed during outcome anticipation including hyperactivity, freezing, vocalization, decreased startle threshold, as well as change in respiratory rhythm or heart rate. Consistent with these known efferent connections of the CeA, electrical or pharmacological stimulation of the CeA was shown to increase blood pressure and heart rate [46;47]. Not surprisingly, lesions of the CeA or its targets result in impaired expression of expectancy behaviors such as fear-potentiated startle [40].

Connections between the BLA and CeA underlie sensory and motor interactions that regulate outcome expectancy learning. Early models of amygdala function during learning proposed that direct connections from the BLA to the CeA underlie behavioral changes observed after conditioning [reviewed in 53;57]. Greater appreciation of the complexity of intra-amygdalar connections point to a lack of direct connections from areas that receive sensory inputs in the BLA (lateral division) with the motor output areas of the CeA (medial division). Updated models have incorporated a largely-ignored group of cell islands found between BLA and CeA nuclei known as the Intercalated Cell Masses (ICM) whose physiology and anatomy have only recently been explored [9;32;93]. ICM neurons receive strong input from the BLA and provide input onto the CeA, thus closing the sensory-motor circuit. In newer models, changes in amygdala function during outcome learning are mediated by this BLA-ICM-CeA pathway [reviewed in 73].

Though much is now known about the cellular and synaptic mechanisms underlying Pavlovian conditioning of fearful stimuli, we are just beginning to understand the basic mechanisms of conditioned expectations for appetitive rewards. Surprisingly, an increasing number of studies indicate some anatomical and physiological differences in amygdalar function in aversive vs. appetitive conditioning. For example, lesions of the amygdala do not block the most basic conditioned responses to appetitive rewards such as approach/orienting to the location of a food reward. Moreover, lesions of the BLA do not block learning of behaviors such as orienting to visual or auditory CSs previously-paired with a food reward. Instead, conditioned responses to visual and auditory CSs are sensitive to lesions of the CeA such as rearing to a visual CS or startle to an auditory CS paired with a food reward [29;60;34]. Nevertheless, recordings from BLA neurons of rats freely navigating through a radial arm maze indicated changes in firing rate just prior to encounter with food rewards found at the ends of arms on the maze [79]. These data reveal an anticipatory signal of reward encoded in the firing of BLA neurons. Interestingly, firing rates of BLA neurons also varied according to the magnitude of expected reward, indicating an ability of BLA neurons to predict upcoming rewards including the sensory or physical features of the outcome. Combined with results described above, these data indicate that BLA neurons are capable of signaling both appetitive as well as aversive outcomes. Similar results have been shown in the primate amygdala [66].

Neurophysiological experiments have also provided additional information about the nature of reward representation in structures interconnected with the amygdala. For example, neurons in the nucleus accumbens, ventral striatum, and prefrontal cortex (PFC) have been shown to respond directly to rewards as well as CSs that predict rewards [1;102;103]. For example, recordings from freely moving rats on a radial arm maze revealed reward-related anticipatory firing in ~30% of neurons samples in PFC [80]. Interestingly, PFC neuron firing rates varied according to the magnitude of the expected reward, indicating that PFC neurons can encode important features of appetitive outcomes similar to that observed in the BLA. Anticipatory firing has also been shown in orbitofrontal cortex (OFC) neurons [21;30;39;110;113] and is consistent with the finding of regional activation (measured by fMRI) of OFC in humans by CSs that predict reward [68].

Early immediate gene expression (IEG; e.g. c-Fos, c-Jun, Zenk) has become a popular tool for the study of neuronal activation during outcome learning [reviewed in 50]. The vast majority

of IEG expression studies have used aversive-conditioning paradigms perhaps because of the rapid learning that can be observed often after a single trial. Following fear conditioning, for example, elevated c-Fos expression is observed in the amygdala [15], with the highest density of labeling found in the BLA and CeA [8;35;63]. Far fewer studies using IEG expression have used appetitive Pavlovian conditioning, with the exception of many studies using drugs of abuse as rewards [22;37;106; reviewed in 50]. Tronel & Sara [114] found increased c-Fos expression in the BLA but not the CeA in rats receiving odor-food pairings. No differences between the BLA and CeA were observed in rats receiving unpaired odor-food presentations. These results suggest that the BLA and CeA are activated during simple conditioning. In addition, using an operant (nose-poke), odor-discrimination task, Hess et al. [38] detected increased c-fos expression in the BLA compared to animals performing an uncued nose-poke task, suggesting an involvement of the BLA in the expectation of cued-rewards. In contrast to the studies described above, not all IEG studies have observed increased expression in the amygdala and still others have shown variable results [92; reviewed in 50]. A number of factors may underlie these varying results including large differences in the behavioral paradigms used and time elapsed before tissue collection and c-Fos detection [see discussion in 50]. Moreover, the presence of IEG expression in neurons of amygdalar nuclei may not definitively reflect a necessary and sufficient role of those neurons in the formation of outcome expectancy. Thus, lesion of the BLA, for example, may not impair learning in certain conditioning paradigms despite the presence of strong IEG expression in the BLA after conditioning.

4. Specific conditions evoke the BLA in the development and maintenance of reward expectancies

There is evidence from a number of procedures that the amygdala is critical for the development of reward expectancies. As describe in detail below, lesions of the BLA produce deficits in Pavlovian second-order conditioning, the reward specific effects of reinforcer devaluation, the Differential Outcomes Effect (DOE), and reward-specific Pavlovian-to-Instrumental transfer [PIT; 11;36;84]. However, lesions to the BLA do not affect acquisition of approach or orientation behaviors elicited by a cue in simple appetitive Pavlovian first-order conditioning paradigm [light cue followed by food dispensed into a food cup; 36;74]. Thus, rats with lesions to the BLA can form general cue-outcome associations; however, their ability to access variations in cue value (imbued from their association with reward) and use such information to guide behavioral choices is impaired. Furthermore, lesions to the BLA do not disrupt unique response-outcome associations measured by an instrumental task that requires two different responses (chain pulling or lever pressing) leading to different rewards [4]. However, these behaviors, and the unique outcomes, were not trained in the same sessions. These differences could be critical in requiring the BLA to contrast specific reward features. The BLA appears to play a more specific role in representing the current or contrasting “affective value of conditioned stimuli” and how this information is used to direct instrumental action [74].

When animals are trained with a second-order Pavlovian conditioning paradigm, BLA-lesioned rats fail to show conditioning to a light that was paired with a tone that had been previously associated with food [36]. That result was taken as evidence that that BLA is involved in transferring specific incentive value from one cue to another. Additional evidence using devaluation procedures [4;36] demonstrates that rats with BLA-lesions are also impaired at re-adjusting their behavior (either operant lever-press/chain pull or Pavlovian orientation/approach responses) to a cue (lever/chain or light) that previously signaled reward but then was recently paired with illness. The behaviors affected are those governed by the cue that was associated with reward—not the devalued reward itself: BLA-lesioned rats will decrease consumption of the reward when presented with the reward alone after devaluation. The data from the Pavlovian devaluation procedure was taken as evidence as the BLA is important for associating cues to the specific current motivational value of the US to which they have been

paired. Similarly, in an instrumental preparation, BLA lesions affect an animal ability to modify representations of the motivational significance of the unique sensory features of reward. Thus, altering sensory-specific representations of appetitive Pavlovian US or instrumental-earned reward depends of the BLA.

The PIT procedure allows for the assessment of the interaction between Pavlovian and Instrumental conditioning. In a PIT study, rats were first instrumentally trained to press two levers: Responses on one lever led to pellet rewards, responses on the other lever led to sucrose water. After that instrumental learning phase a Pavlovian conditioning phase occurred in which two auditory cues were paired with those same outcomes. In the final transfer phase the two auditory cues were presented with the opportunity to lever press. Successful transfer would be if the rat chose the lever that shared the same reward as the cue. Rats with lesions to the BLA were unable to transfer the Pavlovian learning onto the instrumental choice behavior when more than one cue was used [19]. However, when a single lever and cue were used lesions to the BLA did not affect transfer [35;42]. The contrast in results between multiple vs. single cue and reward in PIT was taken as evidence that the BLA is critical for the controlling the selective, but not general, cueing effects related to the unique identity of the reward.

The fact that the BLA is important for the unique sensory features of reward is supported by studies demonstrating that removing the BLA prior to acquiring a discrimination task eliminated the expression of the Differential Outcomes Effects (which was described in a previous section as the enhancement of instrumental discrimination learning when discrete cues are associated with distinct rewards) throughout training with no recovery of function of this phenomenon [11;84]. This procedure differs from the one in which BLA did not affect instrumental training of lever press/chain pull with unique rewards [4] in two ways (1) multiple stimuli and responses were trained together in the same session, (2) there were unique cues that signaled which response would be correct on a given trial. Thus, it is likely that the BLA lesion disrupted performance on a task with the DOP because of the increased cognitive load of multiple unique S-R-O associations to monitor and use within a session. In this recent paper we [84] also noted that damage to the OFC disrupted performance of rats trained with the DOP on a matching-to-position task during the maintenance, but not acquisition, phase of training. In contrast, destruction of the nucleus accumbens core facilitated the development of the DOE (see Figure 5). Such results suggest unique roles for these structures for processing reward expectancies.

All the data together suggest that BLA is critical for the cue-specific evoked sensory representations of outcomes, but not the more general “reinforcing aspects” of outcomes [42; 19]. One hypothesis is that the BLA mediates associations between predictive stimuli (CS) and the unique sensory properties of a reward outcome [3]. Thus, the BLA appears to be needed for learning to distinguish cue associated differences in reward value or for transferring reward value from one cue to another. These processes involve the organism evoking a representation of reward upon presentation of a cue. Specifically, the cognitive loss observed after BLA damage is that organisms are unable to effectively learn to use the memories of associative relationships between cues and specific qualities of a reward event (reward expectancies) to guide behavioral choices.

However, there are some data that suggest that once reward expectancies are formed, the BLA is no longer an important for maintaining reward expectancies in memory or updating them with new information [77]. These results are based on studies that assessed responding to a CS (light) that was paired with food, the BLA was then lesioned, after which the reward was devalued by pairing it with illness and there was a probe test to assess responding to the altered value of CS [77]. With this post-lesion procedure lesions to the BLA did not disrupt the spontaneous decrease in conditioned responding as a function of reinforcer devaluation (but

lesions to the OFC do disrupt this conditioned responding). It was concluded that OFC, but the BLA is needed for maintaining and updating conditioned cue associations with sensory features of reward [77].

However, using a working memory task, a recent paper from our laboratory [96] demonstrated that the **BLA is critical** to maintain and distinguish multiple reward expectations even when the S-O associations were formed prior to alterations of the BLA. Specifically, rats were trained on a matching-to-position (MTP) operant task that has either differential outcomes (food pellet, sweet water) associated with cue levers (left, right) for correct responding (DOP) or random presentation of reward for correct responding (NOP). Once rats met criteria on a non-delayed MTP procedure, they were transferred to the delayed task (DMTP) for 6 days, after which both groups underwent surgery for bilateral cannulae implantation aimed at the BLA. A week after surgery, animals were re-trained for an additional 6 days of DMTP. Saline and two doses of muscimol (0.125, 0.0625 ug/ul) were infused on different days (counter-balanced). We found that muscimol infused into the BLA had no effect in animals trained with the NOP. However, in rats trained with the DOP, muscimol administered into the BLA dose- and delay-dependently impaired memory performance. Such that the DOE is was no longer present in the DOP-trained rats. Thus, the temporary inactivation of the BLA by muscimol eliminated the DOP-trained rats ability to use reward expectancy to guide behavior and, therefore, their behavior then looked like rats trained with the NOP (see Figure 3). Given that the DOP-trained rats performance with an inactive BLA looked like normal NOP-trained rats, one could assume that without the BLA DOP-trained rats could only solve the task by remembering which lever occurred during the sample phase. Thus, even after over training of unique cue-outcome association, an intact BLA is needed for a rat to use unique reward expectations in a working memory task.

It should be note that our study involved more than one S-O association within a session and maintaining different S-O associations across delay intervals on different trials is a complex cognitive task that appears to require the BLA even after S-O associations are well-learned. Furthermore, in the operant DMTP task with the DOP, there was the opportunity for the interactions between Pavlovian-Instrumental associations. Thus, under situations when there is more than one reward expectancy and/or the reward expectancy that guides behavioral choices can change from trial to trial and rather quickly (moments) the BLA continues to be important in modulating learning and memory performance that has become dependent on unique reward expectancies. Previous research has shown that such variables are important for changing the type of association that gains control of behavioral responding [116] and evoking the BLA [see 19]. Furthermore, neither simple Pavlovian conditioning [36;74] or operant discrimination learning [4] is disrupted by lesions of the BLA, but the interaction of these associations is altered after BLA lesions [11;84]. It has also recently been shown that post-training BLA lesions abolished outcome-selective transfer in a PIT task [69]. Those authors reached the same conclusions that we did: The discrepancies on the role of the BLA in continued, after initial acquisition of S-O association, of reward expectations are due to procedural differences that likely result in different types of associations being formed. Two issues that appear to be critical for the BLA's continued involvement in reward processing are: (A) The S-O associations are needed to guide goal-directed behavior; and (B) The number of S-O association. When more than one reward and/or cue is used, this creates a more sensitive behavioral assay of outcome encoding than the single-outcome task. Thus, a key feature in evoking the BLA maybe the specific degree of detail required to discriminated between multiple the reward expectancies. It should be noted that there is evidence that other structures (prefrontal cortex, nucleus accumbens), that are integrated with the amygdala, may contribute in unique ways to how reward expectancies guide instrumental action.

5. Summary

Reward expectancies can be evoked to direct behavior under a range of circumstances. Environmental conditions set the stage for the type of associative relationships that an organism will form between stimuli, responses and outcomes. Data suggest that several interconnected brain structures-- the basolateral amygdala (BLA), the orbitofrontal cortex (OFC), and the nucleus accumbens (NA) are part of the neural circuitry that is integral for cues that predict features of reward and goal-directed behavior [11;20;16;41;84;96]. Substantial evidence suggests that the BLA is integral for associating cues with the sensory features of reward – and how these cue-driven reward expectancies are contrasted from each other or previous states which led to the adaptation of behavior [6;16;42].

The OFC is a cortical region with extensive connections to the BLA whose function may be to determine the goal-directed behavioral correlate of reward expectancy in the presence of a discriminative stimulus [16;42;61;76;101]. More specifically, it has been inferred that the OFC specializes in processing features essential for the guidance of behavior based on outcome expectancies [100]. The BLA and OFC appear to process different aspects of reward information. Whereas the BLA may be responsible for cues being associated with current sensory status of the reward or the contrasting of reward features; the OFC seems to guide responses on the basis of the BLA mediated expectancies [42;77;84;104;100].

Emerging findings suggest that the BLA and OFC form a reward circuit that is necessary for the production of the reward expectancies and the use of these reward expectancies to guide behavior [11;23;61;84]. Furthermore, there is data from paradigms that require attention to multiple S-O associations to guide goal-directed behavior that the BLA and the OFC continue to be critical for using unique reward expectancies to guide goal directed behavior. Collectively, these findings support the idea that the interactions between Pavlovian-induced reward expectancies can change both behavioral and brain processes

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A. Associative relationships for the three term contingency in Two-Process Theory. (Note: S=stimulus, R= response; O=Outcome).

B. With extended training, the cue(s) evoke the reward expectation(s) that can then drive goal-directed behavior

1. Initially, the stimulus cue solely drives behavior:

2. After several pairings, the stimulus cue evokes a reward expectancy that can modulated goal-directed behavior:

Figure 1.
Schematic of associative mechanisms that modulate goal-directed behavior.

Figure 2.

Summary of studies showing that the DOP reduces or eliminates behavioral impairments in multiple models of memory dysfunction.

Figure 3.

Summary of pharmacological studies demonstrating that performance can be disrupted in the DOP condition if the GABA agonist Muscimol is delivered in to the third ventricle (top figures) or the basolateral amygdala (bottom figures).

Figure 4.

Cytoarchitecture of the rodent amygdala. Nissl staining (Left panels) and schematic illustration (Right panels) of the amygdala, general nuclear divisions (bottom left), and detailed nuclear subdivisions (bottom right). Boxed area in top right shown at higher magnification in bottom row. All images take from Allen Brain Atlas (www.brain-map.org). Abbreviations of images in bottom row: amc, amygdalar capsule; <http://mouse.brain-map.org/legend.doBLA>, Basolateral amygdalar nucleus; BLAa Basolateral amygdalar nucleus-anterior part BLAv, Basolateral amygdalar nucleus-ventral part; BMAp, Basomedial amygdalar nucleus-posterior part; <http://mouse.brain-map.org/legend.doCeA>, Central amygdalar nucleus; CEAc, Central amygdalar nucleus-capsular part; CEAl, Central amygdalar nucleus,-lateral part; CEAmed, Central amygdalar nucleus-medial part; ec, external capsule; EPd, Endopiriform nucleus-dorsal part; EPv, Endopiriform nucleus-ventral part; IA, Intercalated amygdalar nucleus; LA, Lateral amygdalar nucleus; MEAd, Medial amygdalar nucleus-anterodorsal part; PIR, Piriform Area; SI, Substantia innominata; st, stria terminalis.

Figure 5.

Summary of data from Ramirez and Savage (2007) demonstrating the selective effects of basolateral amygdala (BLA), orbital frontal cortex (OFC) and nucleus accumbens core (NAc) lesions on the expression of the differential outcomes effect (DOE).

Legend: *Early* refers to the first 2 sessions of training, *Middle* refers to session 10, whereas *Late* refers to sessions 19 and 20 of training.