Invited Address

Synapses, Circuits, and the Ontogeny of Learning

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ABSTRACT: *This article summarizes the proceedings of a symposium organized by Mark Stanton and Pamela Hunt and presented at the annual meeting of the International Society for Developmental Psychobiology. The purpose of the symposium was to review recent advances in neurobiological and developmental studies of fear and eyeblink conditioning with the hope of discovering how neural circuitry might inform the ontogenetic analyses of learning and memory, and vice versa. The presentations were: (1) Multiple Brain Regions Contribute to the Acquisition of Pavlovian Fear by Michael S. Fanselow; (2) Expression of Learned Fear: Appropriate to Age of Training or Age of Testing by Rick Richardson; (3) Trying to Understand the Cerebellum Well Enough to Build One by Michael D. Mauk; and (4) The Ontogeny of Eyeblink Conditioning: Neural Mechanisms by John H. Freeman. Taken together, these presentations converge on the conclusions that (1) seemingly simple forms of associative learning are governed by multiple "engrams" and by temporally dynamic interactions among these engrams and other circuit elements and (2) developmental changes in these interactions determine when and how learning emerges during ontogeny.* © *2007 Wiley Periodicals, Inc.*
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INTRODUCTION TO SYMPOSIUM PROCEEDINGS

Interest in the neurobiology of learning and memory has a long history. Great progress has been made during the past several decades toward understanding the underlying neural basis of associative learning, though many questions still remain. The relative number of developmental studies addressing the neurobiology of learning and memory is, however, comparatively sparse. And unfortunately, there continues to be a lack of integration and communication among researchers in the field of neurobiology and those specializing in development. This lack of communication has hindered the sharing of techniques and research findings, although that is slowly changing (e.g., Fanselow & Rudy, 1998). There clearly needs to be more active effort among researchers to foster

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what can be mutually beneficial exchanges among those with broadly defined interests in development and neurobiology. This symposium was an attempt to bridge this noticeable gap by bringing together experts within their respective fields, encompassing two of the main areas of study in associative learning (fear conditioning and eyeblink conditioning) with a focus on addressing contemporary questions about the development and function of neural systems governing these forms of learning.

Fear and eyeblink conditioning are the two most extensively studied systems in the analysis of the neural basis of associative learning. They are both forms of Pavlovian, or classical, conditioning although they differ in a number of important respects. Pavlovian conditioning involves presentations of a neutral stimulus (conditioned stimulus; CS) in conjunction with a biologically relevant event (unconditioned stimulus; US). Because of the high degree of experimental control over the presentation of the stimuli used, and the stereotyped nature of the conditioned responses (CR) elicited, Pavlovian conditioning has been widely used to study the neural basis of learning and has contributed substantially to the identification of sites of CS and US convergence in the brain. In Pavlovian conditioned fear, the CS is typically paired with a footshock US. Fear is rapidly acquired, being asymptotic after only a few CS–US pairings, and engages multiple response systems (changes in general activity, autonomic function, and potentiation of motor reflexes) that are generally assumed to reflect an acquired emotional (fear) state. The neural structure most implicated in fear conditioning is the amygdala. In contrast, eyeblink conditioning involves presentations of a CS that is typically much briefer in duration than that used in fear conditioning experiments, and that is paired with a more localized US, such as periorbital shock or a puff of air to the eye. Eyeblink conditioning typically requires many more trials than fear conditioning (100–200 CS–US pairings) and results in the expression of a precisely timed, adaptive response—closure of the eyelid. This well-timed, adaptive response requires cerebellar circuitry. The neural substrates underlying learning and memory that are described from research using these paradigms have been relatively well delineated, although current research using anatomical, pharmacological, physiological, and developmental techniques continues to advance our knowledge of brain plasticity and memory storage. It is of interest that the neural systems governing learned fear and eyeblink conditioning are for the most part separate and non-overlapping; yet, there are many parallels in the mechanisms of learning and brain plasticity within these systems (see Reference Medina, Repa, Mauk, & LeDoux, 2002 for review). Developmentally, even within one of these learning situations, the individual components of the system do not all become functional at the same age (see Reference Hunt & Campbell, 1997; Stanton, 2000 for review).

Although most models of fear conditioning assume serial circuits within the brain, it is as yet unclear how many sites of plasticity along this circuit interact to form long-term memories. Historically, within the fear system, acquisition (integration) of information has been assumed to occur within the amygdala, and plasticity in the basolateral nucleus of the amygdala was thought to be not only necessary, but sufficient, for acquisition. More recent work, however, suggests that plasticity in other areas downstream from the basolateral nucleus may also be necessary (Goosens & Maren, 2003; Wilensky, Schafe, Kristensen, & LeDoux, 2006), including plasticity within midbrain and brainstem sites that are critical for response generation. For eyeblink conditioning, several different regions of the cerebellum are involved, including deep nuclei and cerebellar cortex. Each of these areas has been shown to undergo learning-dependent changes in synaptic plasticity, and changes in concurrent activity in these regions, as well as feed-forward and feed-back connections to and within the cerebellum, appear necessary for the acquisition and expression of the eyeblink CR.

The review articles below represent summaries of talks presented as part of a symposium titled ''Synapses, circuits and the ontogeny of learning'' chaired by Mark Stanton and Pamela Hunt. The symposium was presented at the annual meeting of the International Society for Developmental Psychobiology in New Orleans, LA. The articles are published in the order that the talks were given, and represent the authors' recent advances and contributions to the study of fear conditioning (Michael Fanselow and Rick Richardson) and eyeblink conditioning (Michael Mauk and John Freeman). The review ends with a brief discussion of the main issues highlighted and a synthesis of how studying these systems in development can contribute to further understanding of the circuitry underlying fear and eyeblink conditioning, as well as how these neural systems change during ontogeny to allow for increasingly sophisticated learning processes during development.

MULTIPLE BRAIN REGIONS CONTRIBUTE TO THE ACQUISITION OF PAVLOVIAN FEAR BY MICHAEL S. FANSELOW

Abstract

An astonishing level of detail is known about the circuits that mediate the acquisition of Pavlovian conditional fear. Considerable attention has been focused on the amygdala, and to be sure, amygdala activity is normally essential for fear conditioning. However, the complete circuit involves frontal cortex, hippocampus, periaqueductal gray (PAG)

and rostral ventral medulla. Activity within all of these regions during training plays a role in the acquisition of fear. Indeed, under certain conditions of overtraining fear can be acquired in the absence of the amygdala.

Overview

Pavlovian fear conditioning has become one of the standard model systems for examining the neural, genetic, and ontogenetic mechanisms that mediate learning processes. Probably the simplest procedure is to place a rat or a mouse into a chamber and give it one, or more, mild electric foot-shocks. Shortly after shock the animal will become immobile, typically in a crouching posture. Because this freezing response is to the chamber cues that have become associated with shock this freezing provides a measure of short-term memory (e.g., Fanselow, 1980). Additionally, if rats are returned to that chamber even 17 months later they will freeze—so the response can be used as a measure of long-term memory (Gale et al., 2004). Both rats and mice engage in freezing when they recall a fear provoking memory and very young animals can engage in this motor pattern. These are some of the features that have led to the wide adoption of this model system.

Investigations from several laboratories (see Reference Fendt & Fanselow, 1999 for review) have led to a generally accepted proposal for a circuit diagram for this learned behavior (see Fig. 1). The assumption is that the multiple and diffuse stimuli that comprise the context arrive at the hippocampus and there undergo further compression to form a unitized ''configural'' representation of the context (Fanselow, 2000). Initially that contextual information is sent from the hippocampus to the frontotemporal amygdala (FTA) (Swanson & Petrovich, 1998), which contains the lateral and basolateral nuclei. In the FTA, the context representation converges with information about the shock (Maren & Fanselow, 1995). If the contextual representation is given a prolonged time to consolidate, on the order of 2 months, the ''configural'' representation becomes stabilized in association cortex and no longer requires the hippocampus for recall (Anagnostaras, Maren, & Fanselow, 1999; Kim & Fanselow, 1992). Because even after a very prolonged consolidation period, contextual fear depends on the FTA, it is assumed that the memory for the emotional experience (i.e., the shock) is permanently stored there (Gale et al., 2004).

Once the FTA is sufficiently activated by contextual cues that have been associated with shock, signals for response generation are initiated at the central nucleus of the amygdala, which has projections to all the individual components that make up the integrated fear response (Davis, 1997; LeDoux, Iwata, Cicchetti, & Reis, 1988). Of

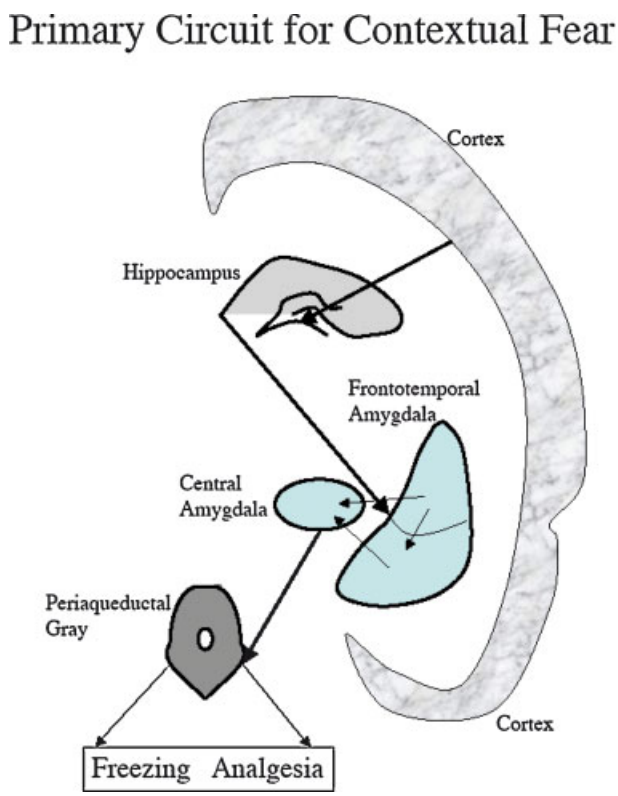

FIGURE 1 The circuit that normally mediates freezing and analgesia produced by long-term contextual fear memories prior to hippocampus-dependent consolidation.

critical importance for freezing are the monosynaptic projections to the ventral PAG (Fanselow, 1991). This pathway descends to motor neurons in the ventral horn of the spinal cord after synapsing in the rostral ventral medulla. A similar descending pathway mediates the analgesic response produced by fear but ends in the dorsal horn of the spinal cord. Fear-induced analgesia regulates the ascending nociceptive information that supports fear conditioning (Fanselow, 1998).

Lesions of the hippocampus, amygdala, and PAG made 1 day after training either severely attenuate or eliminate freezing to shock-associated contextual cues (DeOca, DeCola, Maren, & Fanselow, 1998; Gale et al., 2004; Kim & Fanselow, 1992; Kim et al., 1993). This has led to a general impression that the circuit in Figure 1 is a simple serial circuit with each synapse being necessary for the behavior. Plasticity, in the form of long-term potentiation, at certain synapses within this circuit is recognized to be important as well. NMDA receptor-dependent LTP in hippocampal CA1 and dentate gyrus is essential to form the configural representation of the context (e.g., Stote & Fanselow, 2004) and within the FTA (Maren & Fanselow, 1995) to associate that representation with shock. Thus, by

the time the information reaches the central nucleus it is often thought that the complete contextual fear memory is assembled and stored and that the remainder of the pathway serves a response selection and generation function. For reasons that will become clear below, I will refer to this circuit as the primary contextual fear circuit.

This view of a serial circuit with a limited number of sites for plasticity is clearly an oversimplification. At the very least, the fact that the hippocampus is not necessary once a long enough consolidation period has passed indicates the presence of parallel pathways for the contextual representation (Kim & Fanselow, 1992). However, it is also clear that parallel pathways contribute to learning and behavior even when hippocampus-dependent consolidation is not an issue (Wiltgen & Fanselow, 2003). These parallel pathways seem to contribute specifically when the primary circuit is interrupted *prior* to learning. The clearest example of this is with NMDA-induced excitotoxic lesions of the dorsal hippocampus, which virtually abolish contextual fear when made 1 day after training (Maren, Aharonov, & Fanselow, 1997). When these lesions are made prior to the same training protocol contextual fear was acquired to the same level as intact controls. This finding has important implications for the circuitry of contextual fear learning. Normally, the hippocampus is an essential component of the circuit and damaging it following learning has devastating effects on memory. However, if the hippocampus is not available when conditioning takes place an alternative route to the amygdala is engaged. It is possible that this route is the same one that is used after hippocampus-dependent consolidation, although that hypothesis has not yet been tested. The parallel pathway bypassing the hippocampus, while capable of supporting normal levels of learning, is probably not as efficient as the primary pathway because pretraining hippocampus lesions seem to always produce a deficit following one-trial but not multi-trial conditioning procedures (Wiltgen et al., 2006).

A similar situation exists with the amygdala. To be sure, either pre- or posttraining lesions of the FTA abolish fear conditioning when normal training procedures are used. However, if amygdala lesioned rats are given very extensive overtraining, 75 trials when asymptotic learning is in about 6 trials, they will acquire contextual fear (Maren, 2001). Importantly, if overtraining is given to an intact animal a posttraining lesion still eliminates fear conditioning, indicating that the pathway that bypasses the amygdala does not come into play when the amygdala is intact. This pattern is most clearly demonstrated using temporary inactivation techniques. We overtrained rats with either the amygdala functional or inactivated by the GABA agonist muscimol (Ponnusamy, Poulos, & Fanselow, in press). During overtraining the learning curve for rats with a functional amygdala reached asymptote in less than five trials. Rats with an inactivated amygdala took 10 times as long to reach a similar asymptote. When tested 48 hr later, all the rats showed equivalent expression of a long-term memory for fear. However, inactivating the amygdala during test abolished fear in the rats trained with a functional amygdala but the inactivation had no effect on performance of the rats trained with the amygdala shut down. The data show that there are two pathways capable of mediating long-term fear memory. Normally, an amygdala-dependent pathway is used. If, and only if, the rat learns without the amygdala does it use an amygdala independent pathway.

Unlike the hippocampus, amygdala lesions made even 1.3 years after training block old memories as effectively as new memories (Gale et al., 2004). Therefore, it does not appear that the overtrained animals are using a ''post-consolidation'' pathway when the amygdala is dysfunctional at the time of acquisition (Gale et al.). It is possible that learning in the overtrained-amygdala damaged rat is mediated by structures such as the bed nucleus of the stria terminalis (Walker, Toufexis, & Davis, 2003), which mediate other forms of anxiety, but again this has not been tested.

This pattern of data suggests that neither the amygdala nor the hippocampus are absolutely necessary for acquisition of contextual fear conditioning as there are conditions under which contextual conditioning is acquired in the absence of either structure. Obviously, no structure in the primary circuit is sufficient for fear memory as lesions at any point along the circuit can interrupt previously acquired behavior. Perhaps, asking the question of necessary and sufficient for a neural system mediating learned behavior is not the proper question. Perhaps we should be asking what regions normally participate in learning the behavior. For that question, contrasts of pre- and posttraining lesions are particularly important.

Another assumption of the standard model of fear conditioning is that once information descends from the central nucleus the changes in synaptic efficacy that mediate learning are complete. However, there is at least a suggestion that plasticity at all the relays between the FTA and the ventral horn (central nucleus, PAG, and medulla) make significant contributions to the learning of fear as temporarily blocking activity or even protein synthesis in these structures *during* conditioning appears to attenuate conditional fear responses when memory is tested well after the manipulation (Bailey & Helmstetter, 1998; Goosens & Maren, 2003; Kocorowski & Helmstetter, 1999; Poore & Helmstetter, 1998; Quinn, Sanders, & Fanselow, 2000; Weber & Richardson, 2004).

From Circuits to Networks

There is a distinct serial circuit that *normally* mediates Pavlovian contextual fear conditioning. At virtually every

point along this circuit activity/plasticity during conditioning seems to be required for later performance. However, animals with damage to regions usually considered critical for this type of learning can acquire context fear, albeit often with diminished capacity.

I believe that this pattern of findings has important implications for how we conceive of the systems responsible for learning and memory. Focusing on specific neural circuits mediating functionally distinct behaviors has led to major advancements in our understanding of learning. This paradigm has been enormously profitable for the analysis of conditioning of fear and eyeblink, the two systems that are the subject of this symposium, and arguably the two best understood learning processes. However, it may be time to move beyond analyses in terms of serial circuits. Learning associations requires the convergence of the different bits of information that are the ''content'' of the association (e.g., the different components of a context or the context stimuli and shock). The brain is best thought of as a network of multiple connections between structures with many alternate routes by which environmental information can generate behavioral actions (Scannell, Blakemore, & Young, 1995; Young et al., 1995). Throughout this network there must be multiple points where the same bits of information converge and these are all points with the potential for plasticity that could support behaviorally realized learning. Some routes through the network will be more efficient (Latora & Marchiori, 2001) and support rapid plasticity. When one such route is strengthened that route may in turn block learning in other candidate routes through the processes that normally regulate learning such as fear-induced analgesia (Fanselow, 1998; Rescorla & Wagner, 1972). The winning routes are those that *normally* support learning and one such route is the primary circuit for context fear. However, if that primary route loses its competitive advantage because of a manipulation prior to learning (e.g., lesion, genetic mutation, or past experience) alternate pathways will assume the dominant position. While those routes may not be as efficient (Latora & Marchiori, 2001) as the primary pathway, they are still effective mediators of acquisition. One approach to unraveling these networks is the strategic contrast of pre- and posttraining manipulations.

EXPRESSION OF LEARNED FEAR: APPROPRIATE TO AGE OF TRAINING OR AGE OF TESTING BY RICK RICHARDSON

Abstract

Previous research has shown that learned fear emerges in a response-specific sequence. That is, conditioned freezing to an aversive CS emerges at a younger age than does conditioned fear potentiation of startle to that same CS. This sequential emergence of learned fear allows us to examine whether the expression of fear memories is appropriate to the animal's age of training or their age at testing. I will discuss a number of experiments that have examined this issue. The findings from these studies have implications not only for our understanding of the development of memory, but also for theoretical and neurobiological models of learning.

Overview

Most laboratory-based studies of learned fear employ Pavlovian conditioning procedures. In these procedures, an initially neutral CS (like a tone or a light) is paired with a naturally aversive US (like shock). Subsequent presentations of the CS then elicit a variety of behavioral responses that are indicative of a state of fear (e.g., elevated blood pressure, potentiated startle responses). Recent developmental studies with rats have shown that learned fear emerges in a response-specific sequence. More specifically, an aversive CS will elicit freezing responses (a species-specific fear response in rats) at a younger age than it will elicit cardiovascular responses, and cardiovascular responses at a younger age than a potentiated startle response (see Reference Hunt & Campbell, 1997 for review).

We have recently started using this response-specific sequential emergence of learned fear to examine a fundamental question concerning memory development. Specifically, we have started to explore the question of whether memory is expressed in a manner appropriate to the animal's age at the time of training or its age at the time of test. In other words, if rats are trained at an age where they express fear via one response but not via another response (e.g., an aversive CS elicits conditioned freezing but not potentiated startle in 16-day-old rats), and then tested at a later age (e.g., 23 days of age) where they can express learned fear via both responses, what does their performance look like?

Expectations

When we started this work, there was little doubt about what the outcome would be. That is, it seemed certain that rats would express their memory in a manner appropriate to their age at the time of test rather than their age at the time of training. The basis for this prediction comes from the currently dominant view of Pavlovian conditioning, which maintains that the animal acquires a stimulus–stimulus (S–S) association as a result of pairing the CS and US. The idea that stimulus–response (S–R) associations might also play a part in Pavlovian conditioning has long been out of favor. The S–S view of Pavlovian conditioning has been the basis of previous research on

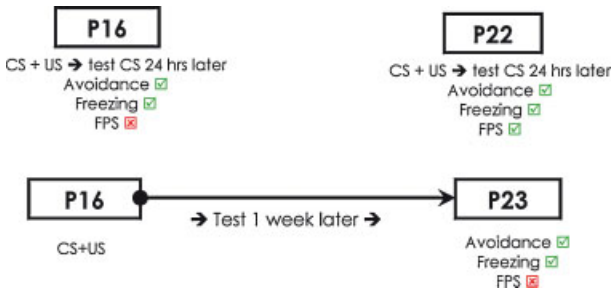

FIGURE 2 Rats given odor-shock pairings at 16 days of age express their learned fear of the odor when they are tested 24 hr later through some behaviors (e.g., avoidance, freezing) but not others (FPS; fear-potentiated startle). Rats trained at 22 days of age and tested 24 hr later express their learned fear of the odor through all three behaviors. The critical finding is that rats trained at 16 days of age and then tested at 23 days of age retain the odor-shock association across the 7-day interval, but they only express their learned fear through response systems that were mature at the time of training (from Reference Richardson & Hayne, in press).

memory in the developing rat (e.g., Johanson & Hall, 1984), and is a key assumption of the models that dominate current analysis of the neural bases of learned fear (e.g., Davis, 1992; LeDoux, 2000). According to these latter models, presentation of an aversive CS elicits a ''central state of fear'' that is instantiated by altered neural activity in the amygdala. How the animal expresses this central state is then largely a consequence of the amygdala activating various downstream structures (e.g., the lateral hypothalamus, the PAG, etc.) that mediate specific behavioral forms of fear (e.g., cardiovascular responses, freezing, etc.).

From the perspective of these neural models, the sequential emergence of learned fear would simply be due to differential functional maturity of the projections from the amygdala to the various downstream structures (e.g., the projection involved in conditioned freezing matures at a younger age than the one involved in potentiated startle). Therefore, as the animal ages the late-developing projection matures, and the animal will now be able to express its ''central state of fear'' via this response. Such a result would provide strong, converging support for findings obtained with lesioned adults (for a discussion of the general notion that developmental studies can provide converging support for studies employing experimentally-induced lesions, see Reference Fanselow & Rudy, 1998). We have explicitly tested this prediction in a number of recent studies.

Results

Using olfactory CSs, we have consistently found that rats express memory in a manner appropriate to their age at the time of training, not their age at the time of test (see Fig. 2 Richardson & Fan, 2002; Richardson, Fan, & Parnas, 2003; Richardson, Paxinos, & Lee, 2000; Yap, Stapinski, & Richardson, 2005). This pattern of results has been obtained with both between-group and within-subject designs. Although this is exactly opposite to what we had expected, such findings have implications not only for our understanding of memory during development but also suggest that our current understanding of what happens during Pavlovian conditioning is somewhat incomplete.

Importantly, Barnet and Hunt (2006) have found the same pattern of results when young rats are trained/tested with a visual CS. That is, learned fear is expressed in a manner appropriate to the rat's age at the time of training, not their age at the time of test. In addition, Simcock and Hayne (2002) reported a conceptually similar result with human infants. Although this latter study did not involve learned fear, it reached the same conclusion about memory expression during development; specifically, ''children's verbal reports of the event were *frozen in time*, reflecting their verbal skill at the time of encoding, rather than at the time of the test'' (p. 229, emphasis added).

Interpretations

Perhaps this pattern of results is only observed during development? For example, Razran suggested that there are two types of learning (a ''higher level'' cognitive type and a ''lower level'' conditioning type) that emerge at different ages (cited in Reference Öhman & Mineka, 2001, p. 513). Perhaps the earlier developing type of learning allows for some contribution from S–R processes while the later developing system involves primarily, if not entirely, S–S processes? This would certainly account for the findings, and also fit nicely with the views of the many developmental psychologists who have suggested that there are qualitative differences in memory processes during development.

However, there are a number of reasons for doubting that these findings are restricted to young animals. First, we are able to reproduce our developmental findings in adult rats by temporarily inactivating particular neural structures at the time of training (Weber & Richardson, 2004). More specifically, if the nucleus reticularis pontis caudalis (the PnC—the part of the brain that mediates fear potentiation of startle) is temporarily inactivated during odor-shock pairings, then adult rats will express their learned fear at a subsequent test (when the PnC is once again active) via freezing but not via potentiated startle. In other words, if the part of the brain that mediates a specific behavioral expression of learned fear is inactive at the time of training (through temporary inactivation or

immaturity), then rats do not appear to subsequently express learned fear via that particular behavior. It must be noted, however, that we do not observe this effect in adult rats when a visual CS is used (see Reference Weber & Richardson, 2004, for a potential explanation). However, there are other studies that also support the idea that inactivating supposed response-generation neural structures at the time of training affects expression of learned fear (see Fanselow's contribution to this article). A second reason for doubting that this finding is restricted to young animals is that there are other reports in the literature illustrating the importance of S–R associations in Pavlovian conditioning with adults (for a recent example, see Reference Donahoe & Vegas, 2004). A third reason for questioning the suggestion that these findings are restricted to young animals is that there are a number of layered network models of learning that postulate that the US is essentially a ''teacher'' that ''designates an effector system in which a CR should appear'' (Kehoe, 1988, p. 412). In other words, if a response system is not active at the time of training, then memory of that experience will not be expressed via that particular response. It seems that it may be time to carefully reconsider our current S–S view of Pavlovian conditioning.

Implications

In addition to raising fundamental questions about what happens during Pavlovian conditioning, the finding that memory is expressed in a manner appropriate to the animal's age at the time of training rather than their age at test also raises important questions about memory and development. For example, this finding once again illustrates that we have to carefully consider how we measure ''memory'' if we train and test animals at different stages of development. Specifically, if we measure at test a response that the animal was incapable of expressing at the time of training, then we may conclude that the animal has failed to remember even though it may have a perfectly good memory of the training experience. In addition, the finding that animal's express their memory in a manner appropriate to their age at the time of training may help us understand certain features of anxiety disorders. In many cases patients realize that their fears are irrational and that they ''should'' feel differently. However, they just cannot. Perhaps they are reacting as they did when they were younger, when they had the experience that has lead to the disorder? Although they are now more mature, they express their memories in a way that is appropriate to their age when they acquired the memory. In that regard, it is interesting to note that Grillon, Pellowski, Merikangas, and Davis (1997), in their work on the dark-potentiation of startle in human adults (i.e., the increase in startle magnitude during the dark compared to the light), found that the magnitude of this effect was not related to self-reported fear of the dark at the present time, but was directly related ($r = .45$) to the subject's self-reported fear of the dark as a child.

Limitations

It is unlikely that all aspects of memory will be appropriate to the animal's age at the time of training, rather than its age at the time of test. For example, Hartshorn and Rovee-Collier (2003) have reported, with human infants, that contextual control of memory is appropriate to the age of testing, not the age at training. Importantly, a reactivation procedure had to be used in that study, and this could have lead to reconsolidation of the original memory at the later age. In other words, the memory of the original training experience could have been reconsolidated in a manner appropriate to the infant's age at the time of reactivation (see Reference Yap et al., 2005 for more detailed discussion of this issue). Whatever the final interpretation of those findings, there is a growing body of evidence showing that the type of *response* emitted at test is often appropriate to the animal's age at the time of training rather than their age at test (i.e., Barnet & Hunt, 2006; Richardson and Fan, 2002; Richardson et al., 2000, 2003; Simcock & Hayne, 2002). This latter work provides a clear foundation for questioning our current views of what is encoded at the time of learning and for our understanding of memory development.

TRYING TO UNDERSTAND THE CEREBELLUM WELL ENOUGH TO BUILD ONE BY MICHAEL D. MAUK

Abstract

Analysis of eyelid conditioning has proven to be very useful for the study of how the cerebellum learns. This utility stems from the relatively direct ways in which eyelid conditioning engages the cerebellum. In essence, this makes it possible to use the extensively characterized behavioral properties of eyelid conditioning as a first approximation of the computational rules for input/output transformations by the cerebellum. These features make it possible to connect specific features of the well-characterized circuitry and synaptic physiology of the cerebellum to specific computational properties. From such studies, which include both experiments and the development of a large-scale computer simulation of the cerebellum, I will suggest basic essential features of cerebellar learning. From these features, certain key questions relevant to development will be posed.

Overview

While caught up in the difficulties of trying to connect brain systems to behavior, and the development of these systems to the ontogeny of behavior, we sometimes overlook that what brain systems do is process information. Because understanding development requires a thorough characterization of the adult endpoint, developmental neurobiology would benefit from an understanding of what a brain system computes and how its neurons and synapses accomplish this computation. Advances in recent years have made it possible to address cerebellar operation in these terms. As will be apparent in the following article by John Freeman, this has facilitated detailed analysis of the developmental changes that lead to the ontogeny of learning mediated by the cerebellum.

A fortuitous combination of factors has contributed to these advances. First, the synaptic organization of the cerebellum is remarkably well characterized, better so than for any other system of the brain. This essentially began in 1967 with the publication of ''The cerebellum as a neuronal machine'' by Eccles, Ito and Szenátothai, which provided a blueprint for the circuitry of the cerebellum. Two years later, David Marr and colleagues (Blomfield & Marr, 1970) initiated a focus of studying the cerebellum in terms of what it computes with his groundbreaking theory of cerebellar cortex. The discovery that several simple and well-characterized forms of motor learning engage the cerebellum in an unusually direct manner has been equally important. In Pavlovian eyelid conditioning for example, conditioned and unconditioned stimuli (CS and US) are conveyed to the cerebellum respectively via the mossy fiber (mf) and climbing fiber inputs, and cerebellar output drives the motor pathways that express the CR. Finally, the rapidly increasing speed and power of computers has made it possible to build detailed computer simulations of the cerebellum. Such simulations can be tested simply by presenting inputs that mimic those occurring during eyelid conditioning. The complex (and well-characterized) behavioral properties of eyelid conditioning provide a rich repertoire of challenges for a cerebellar simulation to meet. The following paragraphs summarize recent work that combines eyelid conditioning experiments and computer simulations to address both what the cerebellum computes, and how.

What the Cerebellum Computes

Because eyelid conditioning maps so directly onto the input/output properties of the cerebellum, the extensively characterized behavioral properties of eyelid conditioning represent a first approximation of *what* the cerebellum computes. The computation revealed, which is feed-forward prediction, ties together three seemingly disparate functions commonly attributed to the cerebellum—coordination, learning, and timing. The properties of eyelid conditioning show that the cerebellum implements learning with timing. When a CS and US are paired at a particular temporal interval, the CR that is acquired is timed to anticipate the US and peak just before its onset. This type of learning is precisely that required for the computation of feed-forward prediction, which is best understood in contrast to feedback control. Feedback control is like a thermostat. Accuracy is accomplished by comparing current performance (temperature) with the goal (thermostat setting) and by making corrections when indicated (activating heater when actual temperature less than goal). Feedback control can be accurate, but only for slow processes since errors are detected and corrected rather than anticipated and prevented. In feed forward control, current conditions are used to predict, based on previous experience, what decisions are required next. A hypothetical feed forward thermostat would predict that the room will get colder when it senses a door has opened and would make an anticipatory activation of the heater. ''Based on previous experience'' means learning, and the associative, temporally specific learning that eyelid conditioning reveals is precisely that required for feed forward prediction. Thus, the cerebellum contributes to motor coordination by making feed forward predictions, the accuracy of which requires associative, temporally specific learning (see Reference Ohyama, 2003; Ohyama, Medina, & Nores, 2002 for reviews).

How the Cerebellum Computes

Recent work using eyelid conditioning experiments and computer simulations suggests the basic blueprint for how the cerebellum implements associative, temporally specific learning. Although there is now ample evidence that plasticity occurs in both cerebellar cortex and deep nuclei, the remainder of this section will review how plasticity is induced in the cerebellar cortex and how this contributes to the observed changes in behavior. This emphasis is based on evidence that lesions of the cerebellar cortex prevent learning and abolish the learned timing of conditioned eyelid responses.

It is well established that the climbing fiber input to the cerebellum acts as a teaching input. How then does this input lead to the acquisition of responses timed to anticipate precisely the arrival of the US? Computer simulations of the cerebellum reveal that climbing fiber controlled learning in the cerebellar cortex is made temporally specific by temporal coding in the granule cell layer. The simulations suggest how different granule cells become active at different times during a stimulus. In this way, the cerebellum cannot only learn to respond to a

stimulus that is consistently paired with a climbing fiber input, it can also learn to respond at the correct time during the stimulus.

The simulations have also shown, however, that the capacity for bi-directional learning (i.e., acquisition and extinction) contributes to the proper timing of cerebellar-mediated learned responses. Essentially, pairing a CS with a US produces acquisition learning for the neurons active toward the end of the CS and extinction learning for neurons active earlier in the CS. In combination, these opposing processes sharpen the timing of the CR so that they are delayed to occur quite specifically just before the US is presented.

Finally, simulations and experiments combined have revealed the fundamental importance of a previously under-appreciated connection: the inhibitory projection of cerebellar output back onto the climbing fibers. This projection allows the cerebellum to regulate its own climbing fiber input to an equilibrium level at which there is no net cerebellar plasticity. Presenting a US disrupts this equilibrium during a CS in favor of more climbing fiber activity. This induces the plasticity that mediates the acquisition of CR. Once responses are well trained, paired CS–US trials produce both strong excitation of climbing fibers (from the US) and inhibition (from the burst of cerebellar output that drives the expression of the CR). If the US is omitted, the strong inhibition disrupts equilibrium of climbing fiber activity in a way that induces the plasticity necessary for extinction. These predictions have been tested empirically, blocking excitatory drive onto climbing fibers causes extinction in animals that are being trained to acquire, whereas blocking inhibitory synapses onto climbing fibers prevents extinction in animals receiving extinction training.

In sum, these studies show the fundamental importance of the ability of the cerebellum to regulate its climbing fiber input. Without this regulation, learning in the cerebellum would go unchecked, occurring even when not necessary. The regulation is necessary for extinction, and by extension, is also therefore necessary for the specific timing seen in cerebellar learning. It is of great interest, therefore, that Freeman and colleagues have shown that maturation of the inhibitory projection from cerebellum to climbing fibers is a key component in the development of cerebellar learning.

ONTOGENETIC CHANGES IN THE NEURAL MECHANISMS OF EYEBLINK CONDITIONING BY JOHN FREEMAN

Abstract

The rate and magnitude of eyeblink conditioning increase substantially between postnatal Days 17 and 24 in rats. Experiments using neuronal unit recording in infant rats during eyeblink conditioning found developmental changes in the activity of cerebellar neurons that correspond to the ontogenetic emergence of eyeblink conditioning. The developmental changes in cerebellar neuronal activity suggest that the ontogeny of eyeblink conditioning is related to changes in learning mechanisms rather than expression mechanisms. Additional neurophysiological and neuroanatomical experiments demonstrated that the developmental changes in learning-related neuronal activity in the cerebellum are due to developmental changes in interactions between the cerebellum and its inputs, the inferior olive and pontine nuclei. Developmental changes in cerebellar regulation of its inputs affect the induction of learning-related plasticity and thereby affect the rate and magnitude of conditioning.

Overview

Eyeblink conditioning has been used as a model system for examining the behavioral and neural mechanisms underlying the ontogeny of associative learning. Eyeblink conditioning is a Pavlovian conditioning paradigm that involves the presentation of a CS such as a tone or light that is paired with an US, which elicits the blink reflex. The conditioned eyeblink response develops as a result of repeated paired presentations of the CS and US. The onset of the eyeblink CR precedes the onset of the US and the peak amplitude of the CR occurs at the onset time of the US. A major advantage of using eyeblink conditioning as a model system for developmental studies of learning is that the behavioral and neural mechanisms underlying eyeblink conditioning have been studied extensively in adult animals (Christian & Thompson, 2003; Gormezano et al., 1983).

Behavioral analyses of the ontogeny of eyeblink conditioning in rats revealed a substantial increase in acquisition of the CR between postnatal Days (P) 17 and 24 (Stanton et al., 1992). Rat pups trained on P17 show very little associative learning, whereas pups trained on P24 show adult-like acquisition and performance. The ontogenetic change in acquisition of eyeblink conditioning is robust and cannot be significantly altered by manipulating experimental parameters that have substantial effects on conditioning such as US intensity, CS salience, arousal level, amount of training, interstimulus interval, or CS modality (Stanton & Freeman, 2000).

The neural systems and plasticity mechanisms underlying eyeblink conditioning in adults have been well characterized (Christian & Thompson, 2003). The intermediate cerebellum, including the anterior interpositus nucleus, cortical lobule HVI, and parts of the anterior lobe play critical roles in acquisition and retention of the

eyeblink CR. The interpositus nucleus is essential for acquisition and long-term maintenance of the CR. Lobule HVI and the anterior lobe are cortical regions that play important roles in initial acquisition of the CR and maintenance of CR amplitude and timing. Information about the CS is sent to the cerebellum by the pontine nuclei and their mf projection to the anterior interpositus nucleus and the cerebellar cortical areas. The cerebellum also sends an excitatory feedback connection to the pontine nuclei. The excitatory feedback connection facilitates activity of the pontine nuclei when the cerebellum is activated during production of the eyeblink CR. The US pathway includes the inferior olive and its climbing fiber projection to the cerebellar nuclei and cortex. The inferior olive also receives feedback from the cerebellum, but the feedback is inhibitory. The inhibitory feedback connection shuts down the inferior olive as the cerebellum is activated during CR production. The inhibitory feedback connection is thought to prevent the induction of redundant or unnecessary plasticity in the cerebellum (Kim, Krupa, & Thompson, 1998; Medina, Nores, & Mauk, 2002).

Our developmental analysis of the neural mechanisms underlying eyeblink conditioning in rodents has used the identified neural circuitry underlying eyeblink conditioning in adults as a neural roadmap for identifying sites of developmental change in the mechanisms of learning. Initial experiments found that disrupting cerebellar development impaired the ontogeny of eyeblink conditioning in rats (Freeman, Barone, & Stanton, 1995; Freeman, Carter, & Stanton, 1995). Subsequent experiments used newly developed methods for recording extracellular neuronal activity in different parts of the eyeblink conditioning circuitry in freely moving rat pups (Freeman & Nicholson, 2000). Our first studies examined developmental changes in the activity of neurons in the anterior interpositus nucleus and cerebellar cortical lobule HVI during eyeblink conditioning in infant rats (Freeman & Nicholson, 2000; Nicholson & Freeman, 2003a; Nicholson & Freeman, 2004). The activity of interpositus neurons and Purkinje cells in lobule HVI can provide a lot of information about the eyeblink conditioning circuitry because they receive inputs from the CS and US pathways, and are the sites of learning-related synaptic plasticity. The activity of neurons in the interpositus nucleus and simple spikes of Purkinje cells in pups trained on P24 was very similar to the adult pattern of activity. The magnitude and temporal pattern of cerebellar neuronal activity correlated very highly with the amplitude and timing of the eyeblink response (Freeman & Nicholson, 2000; Nicholson & Freeman, 2004). In contrast, in pups trained on P17, fewer cerebellar neurons showed learning-specific activity profiles and the neurons that did show learning-related profiles showed a less robust increase in activity relative to the neurons recorded from pups on P24. The developmental change in learning-related activity in the cerebellum suggests that the development of eyeblink conditioning is related to a developmental change in learning mechanisms rather than mechanisms underlying

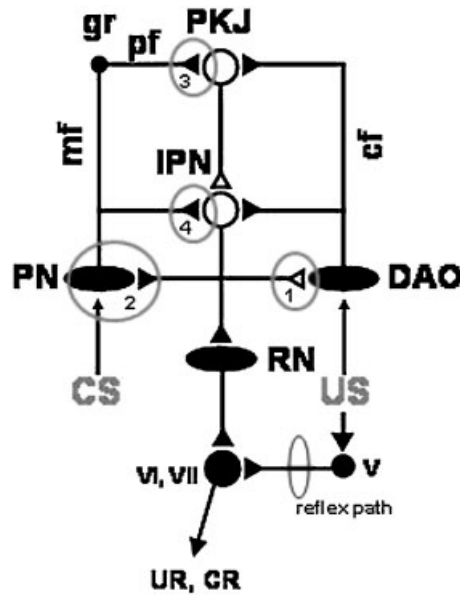

FIGURE 3 Developmental changes in the neural mechanisms of eyeblink conditioning. The cerebellar interpositus nucleus (IPN) and Purkinje cells in the cerebellar cortex (PKJ) receive input from the conditioned stimulus (CS) and unconditioned stimulus (US) pathways. The CS pathway includes the pontine nuclei (PN), the mossy fiber (mf) projection to the IPN and granule cells (gr) in the cortex, and the parallel fiber (pf) projection to PKJs. The US pathway includes inputs to the dorsal accessory division of the inferior olive (DAO) and the climbing fiber (cf) projection to the IPN and PKJs. The pathway for performance of the conditioned response (CR) includes the IPN projection to the magnocellular red nucleus (RN) and its projection to brainstem motor nuclei (VI, VII), which activate eyeball retraction and eyelid closure. The unconditioned response (UR) is elicited by activation of trigeminal nuclei (V), which then activate VI and VII. Filled triangles indicate excitatory synapses and open triangles indicate inhibitory synapses. Gray circles indicate neuroanatomical sites of developmental change that affect the ontogeny of eyeblink conditioning. (1) Inhibitory feedback to the DAO increases with age. The ontogenetic increase in cerebellar inhibition of the DAO results in a decrease in spontaneous and stimulus-elicited cf activity, which alters the induction and maintenance of synaptic plasticity in the cerebellum. (2) Pontine neuronal responsiveness to the CS increases with age. (3) pf input to PKJs increases with age. (4) mf input to the IPN increases with age. Note that 3 and 4 are affected by 2. Weaker CS pathway inputs combined with an unregulated DAO leads to weaker synaptic plasticity in the cerebellum (IPN and PKJ), and weaker conditioning in younger rats.

motor performance. Electrical stimulation of the interpositus nucleus was also used to show that the threshold for eliciting an eyeblink response through the output pathway of the cerebellum does not change between P17 and 24, suggesting that the P17 rats were capable of producing the eyeblink response (Freeman & Nicholson, 2000).

Analyses of neuronal activity from the cerebellar cortex and interpositus nucleus found that in addition to showing developmental increases in learning-related activity, there were developmental increases in the magnitude of activity elicited by the CS and US. Developmental changes in the strength of stimulus inputs to the cerebellum could play a critical role in the ontogeny of eyeblink conditioning by limiting the induction of synaptic plasticity (Fig. 3).

Developmental changes in the US pathway have been examined by monitoring the activity of neurons in the inferior olive and climbing fiber-induced complex spikes in the cerebellar cortex. Neurons in the US pathway show stronger responses to the US in pups trained on P17 relative to pups trained on P24. The developmental decrease in US pathway responsiveness initially seemed to be paradoxical. Why would there be weaker learning in younger rats when their US pathway is more responsive? The key to answering this question was to examine the development of interactions between the inferior olive and the cerebellum. Experimental and computational studies of the neural mechanisms of eyeblink conditioning indicated that the inhibitory regulation of the inferior olive by the cerebellum is critical for induction and maintenance of synaptic plasticity in the cerebellum, which are necessary for learning and long-term retention of the CR, respectively (see Mauk contribution to this article; Medina, Nores, et al., 2002). Detailed analyses of complex spike activity, drug infusions, and quantitative electron microscopy were used to show a substantial developmental increase in inhibitory feedback from the cerebellum to the inferior olive (Fig. 3; Nicholson & Freeman, 2003a; Nicholson & Freeman, 2003b). Developmental changes in climbing fiber activity are due to an ontogenetic increase in the number of inhibitory synaptic connections from the cerebellum to the inferior olive. The ontogenetic increase in synaptic inhibition of the inferior olive produces a developmental transition from an overactive US pathway at P17 to a precisely regulated US pathway at P24. An overactive US pathway results in weaker conditioning (see Mauk contribution to this article), as seen in pups trained on P17.

We have begun an analysis of developmental change in the CS pathway by monitoring the activity of neurons in the pontine nuclei during eyeblink conditioning in infant rats (Freeman & Muckler, 2003). A subset of neurons within the pontine nuclei exhibited developmental changes in the level of spontaneous and CS-elicited activity. The ontogenetic change in spontaneous and CS-elicited pontine activity may be related to developmental changes in excitatory feedback from the cerebellum and motor nuclei efferent to the cerebellum. Developmental changes in pontine responsiveness to the CS may play a critical role in the ontogeny of eyeblink conditioning by influencing the strength of synaptic input to cerebellar neurons and thereby affecting the induction of learning-related plasticity during conditioning (Fig. 3).

Eyeblink conditioning is acquired through induction of synaptic plasticity in the cerebellar interpositus nucleus and cerebellar cortical regions. Induction and maintenance of cerebellar synaptic plasticity depends on interactions between the cerebellum and its afferent input from the pontine nuclei and inferior olive. Feedback from the cerebellum to the inferior olive plays a particularly important role in maintaining learning-related synaptic plasticity in the cerebellum by regulating spontaneous and stimulus elicited activity in the US pathway. Evidence presented here suggests that the ontogeny of eyeblink conditioning depends on the development of interactions between neurons in the cerebellum and the afferent nuclei. The developmental increase in inhibitory synaptic feedback from the cerebellum to the inferior olive influences the induction and maintenance of cerebellar synaptic plasticity during conditioning. Developmental changes in the responsiveness of pontine neurons to the CS have also been observed. The developmental changes in the US and CS pathways combine to influence the induction and maintenance of synaptic plasticity in the cerebellum, and thereby regulate the development of associative learning.

FORGING FUTURE COLLABORATIONS

Fear Conditioning

The findings discussed by Michael Fanselow and Rick Richardson make clear the role of alternative circuits in fear learning. The lesion data with adult animals presented by Fanselow has questioned the assumptions inherent in the serial conditioning model and suggested the idea that there are alternative routes to learning. From the analysis of fear conditioning in developing animals, Richardson has drawn a similar conclusion that suggests that regions involved in response generation may also play an important role in the memory encoding process. The analysis of the neural basis of fear has, and we think incorrectly, separated the systems involved in learning from those involved in the expression (response generation) of acquired fear. It has been assumed that neural plasticity occurs within the basolateral amygdala, but that

amygdala projections to the varied response generation areas does not involve a ''learning'' component (i.e., neural plasticity, broadly construed). It is likely that contiguous activity all along the circuit, from areas involved in sensory detection through those responsible for response generation, are crucial to the encoding process in fear conditioning. A clear demarcation between neural structures and circuits involved in ''learning'' versus those involved in ''expression'' leads to research questions that are limiting our ability to develop a full understanding of the neural networks governing conditioned fear.

Lesion studies in adult animals that are conducted under the general rubric of the long-held serial model, and assumptions about plasticity occurring no further along this circuit than the amygdala, may be misleading. Interrupting the circuit at any level will interfere with putative neural plasticity within structures that are downstream from the site of the lesion. For example, lesions of the basolateral amygdala, which have been shown to block ''acquisition'' of fear may also necessarily prevent activity-dependent synaptic plasticity in amygdala efferents such as the PAG or the pontine nuclei (e.g., PnC). To conclude that the basolateral amygdala lesion interferes with fear conditioning by blocking plasticity within the amygdala that is necessary for learning discounts the notion that these lesions block changes in activity in response generation regions *that may also be necessary for learning*. Our assumptions about the mechanisms underlying fear conditioning, including the prevailing view that Pavlovian conditioning is entirely governed by S–S mechanisms, have guided our analyses of the neural systems involved. Viewed in a different light, that Pavlovian conditioning may additionally involve S–R learning processes, will open new avenues for future research into the neural systems that normally operate in learning. Major questions regarding the nature of what is encoded during Pavlovian conditioning trials, and how memory for these events is governed by complex and interacting neural networks, should be at the forefront of current research into the neurobiological basis of associative learning.

Eyeblink Conditioning

Some of the pioneering work in eyelid conditioning is being conducted in the laboratories of Michael Mauk and John Freeman. As Mauk reminds us in his opening paragraph, we sometimes forget that what the brain does is process information. Studying the details of parallel and serial pathways in the brain can sometimes obscure the global processing characteristics of the nervous system. The cerebellum is involved in complex processes of learning and timing. The analogy presented by Mauk, of the cerebellum being a feed-forward predictor of future events, is a memorable one that captures the complexity of the processes needed for appropriately timed eyelid CRs. What is particularly interesting is the work showing that inhibitory US inputs are involved in shaping the CR, and it is the relatively late maturation of these inhibitory connections that seem to account for the late ontogenetic emergence of eyelid conditioning. Freeman's work has carefully eliminated alternative explanations for this late maturation of eyelid conditioning. It appears as though the young rat is actually *more* responsive to the US, and yet shows substantially reduced capacity for learning. The inhibition of the cerebellar cortex that is required for well-timed eyelid responses is not present in the young animal, and it is the maturation of inhibitory neural pathways that can account, at least in part, for the late ontogenetic emergence of this form of Pavlovian conditioning. Well-timed eyelid responses involve processes of both excitation and extinction, and the young animal may be especially deficient in extinction processes. That is presumably how an overactive US pathway results in weaker eyelid conditioning. This apparently is very different from what we currently know about the fear system.

Conclusions

Eyeblink and fear conditioning are the two most extensively studied systems for analyzing the neural basis of associative learning. While the basic circuits necessary for eyeblink and fear conditioning are to some extent separable (e.g., cerebellum vs. amygdala), the processes that occur within these regions may be highly similar. As Mauk has noted in his article, the examination of developmental changes in associative learning and developmental constraints on learning can be informed by a detailed understanding of how the system functions in adults. Likewise, results from studies using a developmental approach to learning can be used to generate novel predictions that can, in turn, inform research questions addressed by those examining the neural basis of learning in adult organisms. The purpose of this symposium was not to review in detail all that is known about the neurobiology of learning within each of these two paradigms, but more generally to highlight the reciprocity of research questions and approaches to studying the neural basis of eyeblink and fear conditioning by experts in these fields. Researchers studying associative learning from varied perspectives, in talking together, can inform one another and oftentimes generate new directions for research programs that contribute to progress within the broader field of inquiry into the neurobiology of associative learning.

NOTES

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