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Research Statement

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My research examines the neural mechanisms of emotion, specifically the neural mechanisms underlying the enhanced arousal observed during learned fear and anxiety. My main experimental subjects are the New Zealand rabbit and the laboratory rat. I use a variety of techniques including electrophysiology, permanent and reversible lesions, electrical stimulation, and behavioral indices of arousal including Pavlovian fear conditioning and fear-potentiated startle in the awake, behaving animal.

**What are the effects of fear-induced arousal on visual information processing?** Anxiety disorders are accompanied by a chronic state of central nervous system hyperarousal, as indicated by potentiated autonomic, somatomotor, and central nervous system measures. A consistent central nervous system measure of hyperarousal in anxious patients is the predominance of increased low voltage fast activity (LVFA) in the resting electroencephalogram (EEG). LVFA is commonly considered to indicate neocortical arousal and is produced by the release of acetylcholine onto cortical neurons. With respect to cortical sensory neurons, this release depolarizes these neurons and renders them more sensitive to afferent sensory input, thereby creating the most efficient mode for the processing of sensory information. Acetylcholine release onto sensory thalamic neurons exerts a similar action. For example, thalamocortical neurons within the visual system's dorsal lateral geniculate nucleus (dLGN) are depolarized by acetylcholine and fire a steady stream of action potentials of increased frequency that is referred to as the tonic firing mode. During periods of drowsiness, as indicated by the presence of slow, delta (1-4 Hz) wave activity in the EEG, thalamocortical dLGN neurons fire in a phasic burst firing mode that does not accurately relay afferent sensory information to the visual cortex.

In the context of anxiety and anxiety disorders, it might be predicted that fear or anxiety, and the arousal that accompanies these emotional states, would be reflected in an enhanced rate of tonic firing in thalamocortical dLGN neurons. I have recently observed that during the presentation of a fear-arousing *acoustic* stimulus dLGN neurons in the rabbit demonstrate an enhanced rate of tonic firing (Cain, Kapp, & Puryear, 2000). In this experiment a Pavlovian discriminative fear conditioning paradigm was used in which a tone conditioned stimulus (CS+) predicted the occurrence of a brief, aversive unconditioned stimulus (US), while a second tone of a differing frequency (CS-) never predicted its occurrence. Recordings from single dLGN neurons during CS presentations revealed that these neurons demonstrated a significantly greater increase in tonic firing during CS+ than during CS- presentations. In addition, those neurons that demonstrated the burst firing pattern prior to the CS+ assumed the tonic firing pattern in response to the CS+. It is important to note that these results demonstrate that a fear-arousing *acoustic* stimulus produces these changes in neurons of the visual system. In essence, these changes represent a fear-conditioned, cellular correlate of arousal at the level of the dLGN. Please refer to the accompanying paper (Cain, Kapp, & Puryear 2000) for a more detailed discussion of these findings.

### **What is the role of the central nucleus of the amygdala in fear-conditioned thalamic arousal?**

My next project attempted to determine the forebrain structure(s) that may contribute to fear-conditioned thalamic arousal, as reflected in the discriminative responses of dLGN neurons during fear discriminative conditioning. The central nucleus of the amygdala (ACe) is a prime candidate due to its extensive projections to brainstem cholinergic neurons that provide the cholinergic input to the dLGN, its contribution to neocortical arousal, and its role in the expression of a variety of conditioned fear responses. In this experiment, thalamocortical dLGN neurons were recorded during electrical stimulation of the ACe. Stimulation increased the firing rate of these neurons and changed the firing pattern to the tonic firing mode in those neurons that demonstrated burst firing prior to stimulation. In addition, stimulation of the ACe elicited LVFA in the EEG. These changes were identical to those observed in response to a fear-arousing stimulus as described above. These findings are consistent with the notion that the ACe contributes to fear-conditioned thalamic arousal by rendering the neurons of the dLGN most sensitive to visual afferent information from the retina and creating optimal conditions for the transfer of this information to the visual cortex. The results of this project are being presented at the Society for Neuroscience annual meeting and a manuscript is currently in preparation. Please refer to the enclosed abstract for a brief report of my findings (Cain, Kapp, & Puryear, 2000).

Based on the above findings, I am attempting to determine if inactivation of the ACe will abolish the conditioned response characteristics of dLGN neurons during discriminative fear conditioning. Muscimol, a GABA<sub>a</sub> agonist, is being used to inhibit the efferent projections of the ACe, which receive a strong GABA innervation. In this experiment, animals receive Pavlovian fear conditioning. Following isolation of a characteristic dLGN neuron, a block of CS+/CS- trials is presented and then either muscimol or saline is infused into the ACe. Following infusion, an additional block of CS+/CS- trials is presented. It is predicted that inactivation of the ACe will abolish the conditioned response characteristics of dLGN neurons. Should this prediction be supported by the results of this experiment, then they will provide additional evidence for the contribution of the ACe to fear-conditioned thalamic arousal.

My future research efforts will establish a more complete understanding of the neural mechanisms that contribute to the fear-conditioned response characteristics of dLGN neurons. Initially, I will attempt to determine if brainstem cholinergic neurons, commonly referred to as the Ch-5 cell group, that innervate the dLGN contribute to these response characteristics by temporarily inactivating them while recording from dLGN neurons in response to CS presentations. To understand the mechanism of this conditioned response, I will examine the relationship between the dLGN, the reticular nucleus of the thalamus, and the ACe. The reticular nucleus of the thalamus provides a potent inhibitory input onto dLGN thalamocortical neurons and is believed to play an important role in thalamic arousal via a disinhibitory process. I will attempt to determine if reticular nucleus neurons demonstrate fear-conditioned response characteristics and determine if the ACe contributes to these characteristics. This analysis will be similar in design to my analysis of the contribution of the ACe to the conditioned response characteristics of dLGN neurons. The ACe is part of a continuum known as the central extended amygdala. I would like to examine the contributions of other components of the continuum, such as the lateral portion of the bed nucleus of the stria terminalis, in altering the processing of thalamic visual information during conditioned fear. In order to complete these projects I will apply for extramural funding, such as to the National Institute of Mental Health, to provide state

of the art laboratory equipment to conduct these experiments and to enable me to teach undergraduates using the latest technologies available in neuroscience to prepare them for graduate school.

**Do Ch-5 cholinergic neurons contribute to conditioned inhibition?** Based on my findings in the above experiments, it is evident that the cholinergic system may help to gate our sensory systems by enabling them to process information most efficiently during periods of enhanced arousal, thereby enhancing an animal's ability to respond optimally to a threatening stimulus. Therefore, it is possible that the cholinergic system also helps to process information that indicates the lack of threat. To begin to address this question, I am examining the effects of permanent and reversible lesions of the Ch-5 cell group in the rat on the conditioned inhibition of fear-potentiated startle. In this paradigm the rat is presented with a conditioned stimulus (light), which is paired with an aversive unconditioned stimulus (mild foot shock). During testing, the animal is presented with the light conditioned stimulus and a standard startle stimulus (white noise burst) or startle stimulus alone in the absence of the light. Potentiated startle occurs if the rat demonstrates a greater startle amplitude in the presence of the light and the startle stimulus together, as compared to the startle stimulus alone. The reduction of fear can also be studied using the fear-potentiated startle paradigm. Rats are given training where a light is paired with an aversive stimulus (mild foot shock) on some trials and on other trials the light is presented with a tone and the light-tone compound is never followed by the aversive stimulus. After training, the light produces fear-potentiated startle when it is presented alone, but fear-potentiated startle to the light is greatly reduced when the tone accompanies the light. The Ch-5 cell group places the startle circuit under a constant state of inhibition. This suggests that the Ch-5 cholinergic cell group may contribute to the inhibition of fear-potentiated startle. Therefore, inactivation of the Ch-5 cell group with permanent or reversible lesions may abolish this inhibition. This contribution of the Ch-5 cell group to the inhibition of fear-potentiated startle is currently under investigation. If the Ch-5 cell group has a role in the conditioned inhibition of fear-potentiated startle, I will design additional experiments to determine the neural circuit involved in the inhibition of fear.

**Involvement of undergraduates:** During my graduate career, I have supervised four independent study students and several work-study students. All of these students were involved with all aspects of data collection and analysis and found it to be a very rewarding experience. The students contributed to the training of the animals, the recording of single neurons, the analysis of the EEG, heart rate, and single neuronal data, the preparation of tissue for histological analysis, as well as making solutions and other general laboratory work. My undergraduate assistants have co-authored abstracts for the Society for Neuroscience annual meeting, co-authored publications, and have attended the Society for Neuroscience annual meeting. The technological advances in neuroscience have simplified the process of data collection and analysis and this allows undergraduates to more fully participate in all stages of the process. I fully expect and will ensure that undergraduates will be able to make significant contributions to all projects being conducted in the laboratory.