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A major problem in attempting to understand complex physiological processes, such as brain neuromodulation, or complex behavioral processes, such as arousal, is finding a simple system that will permit such analyses. The brain stem masseteric (jaw closure) reflex in cats is such a system. It is simple, containing only one synapse in brain, and receives dense inputs from two neurochemical systems important in neuromodulation and arousal. Initial pharmacologic studies showed that locally applied norepinephrine facilitated the reflex response. More importantly, physiologic conditions, known to activate the brain norepinephrine system, also facilitated the response. This latter finding was shown to be causal, rather than correlative, by a study which found that the facilitation could be blocked by prior destruction of the norepinephrine input specifically to the reflex circuitry. These data represent the first definitive example of an activational effect in an intact and behaving organism being attributable to a particular central neurotransmitter acting at a specific brain site.

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ANNUAL TECHNICAL REPORT

At the time of this report, we are nearing the end of our fifth year of support from the AFOSR. The first three years of this support (AFOSR 85-0034) were aimed at studying mammalian bioreactivity, with the explicit goal of examining NE neurons in the locus coeruleus (LC) of behaving cats under physiologically and/or ecologically relevant conditions. This work is now virtually completed except for one study on LC neuronal activity during the feline defense reaction.

These studies may be summarized by saying that the activity of LC-NE neurons in behaving cats changes in direct relation to behavioral state (sleep-wake-arousal). They are silent in REM sleep, moderately active during waking (1 spike/s), and strongly activated during stress/arousal (3-10 spike/s). More specifically, we found that stimuli that were simply arousing, i.e. capable of eliciting an orienting response, activating, etc., evoked only a phasic increase in LC activity, lasting at most for several hundred milliseconds or a few seconds. By contrast, when cats were exposed to challenging or stressful stimuli (as indicated by autonomic activation), a tonic elevation of LC neuronal activity was produced. It did not matter if the stressor was environmental (e.g. loud noise) or physiological (e.g. glucoprivation), the same 2-4 fold increase in activity was observed. We believe, as do other investigators in this field, that the phasic response of this system to salient stimuli is consistent with their hypothesized role in attention and response preparedness. On the other hand, their tonic activation, exclusively in response to stressful stimuli that may represent challenges to the integrity of the organism, indicates that they constitute part of an emergency system operating in this temporal domain.

The publications deriving from this work are listed in the bibliography at the end of this section. Additionally, a copy of a review chapter, which summarizes this work ("Locus coeruleus neuronal activity in behaving animals", in: The Pharmacology of Noradrenaline in the Central Nervous System, Heal and Marsden, eds.), can be obtained from Dr. William Berry of the AFOSR.

We are currently at the end of the second year of support on a three year AFOSR grant (87-301) which was a renewal of the original three year award. This grant took the results from the first grant, dealing with LC unit activity, as a starting point for two new initiatives. Both questions concern the aftermath or consequences of neuronal activation. I.) In the first series of studies, we sought to identify a discrete neuronal circuit whose output in physiology or behavior could be used to gauge changes in central chemical neurotransmission. II.) The second series of studies was directed at determining whether neurotransmitter release in brain is tightly coupled to neuronal activity.

I.) The masseteric, or jaw closure, reflex is a simple component of behavior (a monosynaptic reflex) that receives known dense neurochemical inputs. Neurons with stretch receptors in the masseter muscle have their cell bodies in the mesencephalic n. of V (MesV) and synapse upon motor neurons in V (MoV) that innervate the masseter muscle. Neurons in the motor nucleus receive dense NE and 5-HT afferent inputs directly on their cell

bodies. The reflex response (jaw jerk) can be measured by placing recording electrodes in the muscle and can be elicited by phasic electrical stimulation of MesV.

We began our studies in this area with pharmacologic experiments involving drug injections (0.5 1) directly into MoV of the cat. Our initial interest was in NE, and we found that its local application facilitated the reflex response for approximately 15 min, in doses as low as 125 ng (Stafford & Jacobs, in press a). Furthermore, it did so by an action at an alpha-1 adrenergic receptor (Stafford & Jacobs, in press a).

In the next series of studies, we examined the effects on this reflex of exposing cats to a variety of environmental conditions that were known to activate the brain NE system, e.g. clicks, loud white noise, and dog confrontation. We found that all of these manipulations augmented the reflex response (Stafford & Jacobs, in press b).

The final experiment in this line of investigation was the crucial one. Could we demonstrate that a cause and effect relationship existed between NE release in response to the various aforementioned conditions and facilitation of the masseteric reflex response? We approached this by specifically denervating the NE input to MoV with the catecholamine neurotoxin 6-OHDA. This was done on one side of the brain, while the contralateral MoV was left intact. The animals were then re-exposed to click, white noise, or dog while the reflex response was examined bilaterally. As hypothesized, upon exposure to these conditions, the reflex on the denervated side of the brain was augmented to a lesser degree (or, in some cases, not at all), while the reflex response elicited on the intact contralateral side continued to display the expected facilitation (Jacobs & Stafford, in press b).

We believe that these data are the first to show that the release of NE, under physiological conditions, can facilitate behavioral output in an unanesthetized, intact organism through a specific action directly upon motoneurons. These data were also the inspiration for a meeting organized by the P.I. on simple systems in vertebrate organisms (published as a book: Modulation of Defined Vertebrate Neural Circuits, New York Academy of Sciences).

Our current line of investigation of the masseteric reflex involves examining the effects of 5-HT in this system. Thus, far, the data indicate that local application of 5-HT in MoV exerts a facilitatory effect on the reflex response similar to that produced by NE. We have not yet studied 5-HT's actions under physiologically relevant conditions.

II.) Over the past 10 years, our single unit studies of brain NE and 5-HT neurons in behaving animals have shown a clear-cut distinction between these two groups. As described above, NE neurons are highly dynamic, dramatically changing their activity in response to a variety of stimuli. By contrast, 5-HT neurons are difficult to perturb by any of a variety of environmental and physiological challenges. These data are contrary to those deriving from pharmacological and neurochemical approaches. For example, there is a large literature indicating that brain serotonin is important for thermoregulation, yet we found that 5-HT neuronal activity was

unaffected by environmental heating or during a pyrogen-induced febrile response. A basic assumption inherent to the usefulness of single unit activity for our purposes is that such activity is tightly coupled to the axon terminal release of neurotransmitter. If that is untrue to any significant degree, it seriously undermines the value of single unit activity as a reflection of the functioning of brain neurochemical systems.

One technique which can be used to investigate the regulation of serotonin release in the behaving animal is in vivo brain microdialysis. In this technique, a small probe with a permeable membrane (m.w. cutoff = 5,000) is inserted into the brain structure of interest while artificial CSF is perfused through the probe. Neurochemicals passively diffuse across the membrane and are collected in the artificial CSF. Measurement of neurotransmitter and metabolite levels collected in this sample over some period of time are thought to reflect extracellular concentrations of these compounds. Parametric analyses and validation of the technique were initially worked out in the rat (Auerbach et al., in press). We then adapted this approach for use in the cat and measured extracellular serotonin in the anterior hypothalamus and caudate nucleus under several conditions identical to those in which we examined serotonergic single unit activity. Thus, we examined the relationship between extracellular serotonin and behavioral state, with the hypothesis that serotonin would increase during periods of arousal, and decrease during sleep, thus paralleling the changes that we have seen with neuronal recordings in behaving animals. Secondly, we examined the possibility of a dissociation between neuronal activity and serotonin release by measuring extracellular serotonin during a thermoregulatory challenge. Since we had found that neuronal activity was not specifically altered during pyrogen induced fever, and the literature has implicated brain serotonin in the regulation of body temperature, pyrogen-induced fever appeared to be a situation in which nerve terminal modulation might play a significant role in the determination of extracellular serotonin release.

High pressure liquid chromatography with electrochemical detection was used for analysis of 5-HT levels. Electrochemical detection was performed by a dual potentiostat electrochemical detector with two parallel working electrodes at 590 and 520 mV relative to an Ag/AgCl reference electrode. This dual electrode system was used to enhance our ability to detect co-elution of unknown substances with serotonin. Thus, for all samples, the ratio of serotonin peak heights at maximal and half maximal potentials was calculated (the current ratio) and compared to the current ratio in pure serotonin standards. This ratio was used to determine, before performing any experimental manipulation, whether the observed peak represented uncontaminated serotonin.

Our first experiment in this series examined the relationship between extracellular serotonin and behavioral arousal. If neuronal activity contributes significantly to the control of serotonin release, extracellular serotonin should increase during periods of increased behavioral arousal, and decrease during sleep periods. We measured extracellular serotonin and simultaneously monitored behavioral state using a set of polygraphic criteria. As predicted, extracellular serotonin increased and decreased in parallel with behavioral state changes. Thus, extracellular serotonin

appears to be controlled at least in part by fluctuations in the neuronal activity of serotonin containing neurons.

We next explored the hypothesis that the release of extracellular serotonin might be modulated independently of neuronal activity by local factors in the nerve terminal region. Thus, we examined release of serotonin in the anterior hypothalamus during fever induced by a synthetic pyrogen. As described above, serotonergic neuronal activity is not significantly changed during fever, and yet a substantial literature suggests that serotonin release in the anterior hypothalamus is increased in response to thermoregulatory challenges. Thus, this manipulation seemed to be a likely candidate for significant contribution of nerve terminal modulation (i.e. one in which serotonin release might be dissociated from the firing of serotonergic neurons).

We administered synthetic pyrogen (muramyl dipeptide, 50 ug/kg i.v.) to six cats while brain temperature, behavioral state, and extracellular serotonin were measured continuously. In no instance did we observe a consistent change in serotonin release that could not be attributed to a change in behavioral state during this manipulation. A time series regression analysis demonstrated that extracellular serotonin was significantly related to behavioral state change consequent to the drug-induced febrile response, and not significantly affected by changes in body temperature per se. Thus, these neurochemical data strongly support the single unit data that were previously gathered in our laboratory.

Most of our dialysis data are contained in a doctoral thesis (Wilkinson, 1989) which can be obtained from Dr. William Berry of the AFOSR. These data are currently being prepared for publication. The bulk of the reflex data regarding NE's effects are "in press" as back-to-back articles in the Journal of Neuroscience (copies also available from Dr. Berry). Dr. Ribeiro-do-Valle, a visitor from Brazil, will soon be writing up our results with 5-HT and the masseteric reflex. In addition, most of these microdialysis and reflex results have been reported in abstracts and chapters (see following bibliography).

PUBLICATIONS DERIVING FROM AFOSR SUPPORT

Book

**Davis, M., Jacobs, B.L., and Schoenfeld, R.I. (Eds.). Modulation of Defined Neural Circuits. New York Academy of Science (in press).

Chapters

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