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19. ABSTRACT (Continue on reverse if necessary and identify by block number) 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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FINAL TECHNICAL REPORT

We have just completed the third year of support on a three year AFOSR grant (87-301) which was a renewal of an original three year award. This grant took the results from the first grant, dealing with locus coeruleus single unit activity in behaving cats, 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 μ l) 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, 1990a). Furthermore, it did so by an action at an alpha-1 adrenergic receptor (Stafford & Jacobs, 1990a).

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, 1990b).

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, 1990b).

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, 1989).

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. A manuscript describing these data is currently being prepared by Dr. Ribeiro-do-Valle, the principal investigator in this study. 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., 1989). 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 release 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 condition 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 coelution 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 μ g/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 was sent to Dr. William Berry of the AFOSR. These data have recently been submitted for publication (Journal of Neuroscience).

PUBLICATIONS DERIVING FROM AFOSR SUPPORT (87-0301)

Book

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Chapters

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Jacobs, B.L., Abercrombie, E.D., Fornal, C.A., Morilak, D.A., Rasmussen, K. and Wilkinson, L.O. Electrophysiological studies of brain noradrenergic and serotonergic neurons in behaving animals. In: Progress in Catecholamine Research, Part B: Central Aspects. M. Snadler et al. (Eds.) Pergamon Press, New York, 1988, 163-167.

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Chapters (continued)

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Journal Articles

- Fornal, C.A., Litto, W.J., Morilak, D.A. and Jacobs, B.L. Single-unit responses of serotonergic dorsal raphe nucleus neurons to environmental heating and pyrogen administration in freely moving cats. Experimental Neurology, 1987, 98, 388-403.
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- Wilkinson, L.O. and Jacobs, B.L. Lack of response of serotonergic neurons in the dorsal raphe nucleus of freely moving cats to stressful stimuli. Experimental Neurology, 1988, 101, 445-457.
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Wilkinson, L.O., Auerbach, S.D. and Jacobs, B.L. Levels of extracellular serotonin are regulated by neuronal activity: parallel changes in extracellular serotonin levels and behavioral state in undisturbed and febrile cats (submitted, Journal of Neuroscience).

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