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The origin and physiological effects of serotonin on neurons within the cat's cerebellar nuclei

Kitzman, Patrick Harvey, Ph.D.

The Ohio State University, 1994
THE ORIGIN AND PHYSIOLOGICAL EFFECTS OF SEROTONIN
ON NEURONS WITHIN THE CAT'S CEREBELLAR NUCLEI

DISSERTATION

Presented in Partial Fulfillment of the Requirement for
the Degree Doctor of Philosophy in the Graduate School of
The Ohio State University

BY

Patrick Harvey Kitzman, M.S.

*****

The Ohio State University
1994

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Graduate Program in Anatomy
DEDICATION

To my parents
ACKNOWLEDGMENTS

I wish to thank Georgia Bishop for allowing me the opportunity to learn from her the past three years and for the incredible amount of patience she has shown for me over that same time. Her dedication has truly been inspirational.

I wish to thank Katharine Dillingham. Her technical assistance and expertise were priceless and have truly been appreciated.

I wish to thank Drs. King and Martin who constantly reminded me that there is more than one way to look at the world and for showing me that learning is a continuous process which must continue throughout life.

I wish to thank Karl Rubin for his photographic assistance.

Finally, I wish to thank my family for all the love and support they have given me the past four years.
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LIST OF ABBREVIATIONS

BC  BRACHIUM CONJUNCTIVUM
BP  BASILAR PONS
CG  CENTRAL GREY
CS  SUPERIOR CENTRAL NUCLEUS
ECN EXTERNAL CUNEATE NUCLEUS
DR  DORSAL RAPHE
DTN DORSAL TEGMENTAL NUCLEUS
FTG GIGANTOCELLULAR TEGMENTAL FIELD
FTL LATERAL TEGMENTAL FIELD
FTP PARALEMNISCAL TEGMENTAL FIELD
GABA $\gamma$-AMINOBUTYRIC ACID
IOC INFERIOR OLIVARY COMPLEX
LC  LOCUS COERULEUS
LLV VENTRAL NUCLEUS LATERAL LEMNISCUS
LRN LATERAL RETICULAR NUCLEUS
MLB MEDIAL LONGITUDINAL BUNDLE
NMDA (N-METHYL-D-ASPARTATE)
NRM NUCLEUS RAPHE MAGNUS
NRO NUCLEUS RAPHE OBSCURUS
NRP NUCLEUS RAPHE PALLIDUS
PRN PARAMEDIAN RETICULAR NUCLEUS
SO  SUPERIOR OLIVE
TB  TRAPEZOID BODY
TRC  TEGMENTAL RETICULAR NUCLEUS
XII N  CRANIAL NERVE XII
5ST  SPINAL TRIGEMINAL TRACT
8-OH-DPAT  (+)-8-1-(2,5-DIMETHOXY-4-IODOPHENYL)-2-
             AMINOPROPA
INTRODUCTION

The coordinated movement of voluntary muscles is the result of complex interactions between several regions of the brain and spinal cord. One of the brain regions involved in controlling motor activity is the cerebellum. The cerebellum is anatomically and physiologically divided into the cortex, which comprises a trilaminar outer mantle, and four nuclear masses defined as the medial, anterior interpositus, posterior interpositus, and lateral nuclei which are located deep to the cortical mantle within the white matter core of the cerebellum.

Overview of cerebellar function.

The role of the cerebellum is not to initiate motion but rather to co-ordinate activity between various muscle groups participating in a movement. Of the two areas of the cerebellum, the cerebellar cortex has been the focus of previous anatomical and physiological research. Classically, the cerebellar cortex is proposed to serve two different functions. The vermal and paravermal regions are postulated as being involved in regulating posture and stereotypic movements (Eccles et. al., 1972; 1979). These regions compare descending motor commands, which have been relayed from the
cerebral cortex via the inferior olive as well as the pontine and the lateral reticular nuclei, with information about the state of an ongoing movement (e.g. information from muscle spindle fibers and joint receptors) which is relayed from spinal levels via the dorsal spinocerebellar tract and the cuneocerebellar tract (Eccles, 1979; Arshavsky et. al., 1983). The cerebellum also receives input from the ventral spinocerebellar tract and the spinoreticulocerebellar pathway which convey information about the activity of central spinal mechanisms (Lundberg, 1971; Arshavsky et. al., 1983). The cerebellar cortex compares these motor cortical and spinal inputs and adjusts motor activity through circuits involving the cerebellar nuclei. The cerebellar nuclei are critical for proper motor function because neurons in these nuclei project to brainstem areas that are directly involved in controlling motor neurons in the spinal cord. The importance of the cerebellar nuclei in regulating motor activity becomes evident following their destruction which results in gross distortions of limb and trunk movements (Gilman et. al., 1981). The fastigial and the interposed nuclei send signals to spinal motor neurons via three pathways; the red nucleus/rubrospinal pathway, the vestibular nuclei/vestibulospinal pathway, and the reticular nuclei/reticulospinal pathway (Gilbert, 1974; Eccles, 1979; Ito, 1982; Bloedel, 1992). In addition, the interposed nuclear neurons send corrective signals back to the motor cortex, via the ventral lateral (VL) nucleus of the
thalamus. A separate function for the olivocerebellar pathway has recently been proposed. It has been hypothesized that the synchronous and rhythmic activity of the inferior olive acts as a pacemaker for the cerebellar nuclear neurons, which in turn produce a rhythmic modulation of the motor systems (Llinas and Welsh, 1993).

The second proposed function of the cerebellar cortex involves the hemispheres which is proposed to influence the cerebral motor cortex to ensure a smooth and orderly sequence of muscle contraction. This portion of the cerebellar cortex receives inputs from the motor association cortex and supplementary motor cortex and send inhibitory projections to the dentate nucleus (Eccles, 1979). The dentate nucleus in turn projects to the motor cortex (via the VL nucleus of the thalamus) which in turn sends projections to the spinal cord to effect movement. In addition, the dentate nucleus projects to the VA nucleus of the thalamus which in turn projects to the association areas of the frontal cortex (Eccles, 1979). Finally, the dentate nucleus send projections to the red nucleus (Courville, 1966) and the medullary reticular formation (Tolbert et. al., 1980). Both of these areas in turn project to the spinal cord to effect force and volition of a movement. More recent studies have proposed that the lateral cerebellum participates in regulating the timing of sequential movements (Ivry et. al., 1989).

In addition to it’s involvement in motor function,
several studies have proposed that the cerebellum may also be involved in several cognitive processes such as spatial and discriminative learning and in the acquisition and retention of rapidly learned autonomic conditioned responses (Lalonde and Botez, 1990; Dahhoui et al., 1992; Supple and Kapp, 1993; Lalonde, 1994).

As stated previously, previous studies have focused on determining the function of the cerebellar cortex. The focus of the present study is on the cerebellar nuclei and in particular the nuclei interpositus anterior and posterior.

**Afferents to the cerebellar nuclei.**

The activity of the cerebellar nuclear neurons has been shown to be regulated by excitatory inputs which originate within the brainstem (Ito et al., 1970; Eccles, 1974; Kitai et al., 1977; McCrea et al., 1977). Some of these inputs are collaterals of the two major afferent systems that project to the cerebellar cortex, named climbing fibers and mossy fibers (Chan-Play, 1973, Qvist, 1989). Specifically, the cerebellar nuclei receive collaterals from axons that originate within the pontine nuclei, the nucleus reticularis tegmenti pontis and the inferior olivary complex (Shinoda et al., 1992; Mihailoff, 1994). Brainstem nuclei that have been shown to give rise to afferents to the cerebellar cortex (e.g. the inferior olivary complex, the lateral reticular nucleus, and the reticular tegmental pontine nucleus) also contain neurons that project only to the cerebellar nuclei (Qvist,
Finally, the cerebellar nuclei also receive inputs from brainstem nuclei that project only to the cerebellar nuclei (Kitzman and Bishop, 1994). Along with the excitatory afferents from the brainstem, the cerebellar nuclei also receive inhibitory inputs which arise from Purkinje cells in the cerebellar cortex (Ito et al., 1964; 1970; Chan-Palay, 1973).

Output of the cerebellum.

The importance of the cerebellar nuclei is evident in the fact that they are the only source of efferents from the cerebellum. All four of the cerebellar nuclei have been shown to project to the inferior olive, the superior colliculus, and the pedunculopontine nucleus (Angaut et al., 1970; Sugimoto et al., 1982; Hazrati and Parent, 1992). In addition, the fastigial nucleus sends projections to all four vestibular nuclei and the raphe nuclei (Walberg et al., 1962a; Asanauma et al., 1983). Both the fastigial and interposed nuclei project to the reticular tegmental pontine nucleus (McCrea et al., 1978; Asanuma et al., 1983) while the interposed nuclei also project to the pretectal area (Sugimoto et al., 1982) and the red nucleus (Angaut et al., 1970). Both the lateral nucleus and the fastigial nucleus project to the medullary reticular formation (Batton III et al., 1977; Tolbert et al., 1980). The lateral nucleus, like the interposed nuclei, also projects to the red nucleus and the ventral anterior and ventral lateral nuclei of the thalamus (Courville, 1966;
Neurotransmission versus neuromodulation.

Neurotransmission refers to the process by which information, in the form of electrical signals, is converted to a chemical signal and carried by one or more messengers (neurotransmitters) from a presynaptic to a postsynaptic neuron. In order for a chemical to be termed a neurotransmitter it must meet several criteria: 1) it must be produced in the neuron; 2) it must be released from the presynaptic terminal; 3) when exogenously applied it must mimic the actions of the endogenously released transmitter; 4) it must have a system for deactivation, either by enzymatic degradation or by some form of uptake system (Schwartz, 1991).

Neurotransmitters, which can be either excitatory (e.g. glutamate and aspartate) or inhibitory (e.g. GABA and glycine), utilize ionotropic receptors that are embedded in the postsynaptic membrane. These receptors are directly linked to or have as an integral part, an ion channel. Upon binding of the neurotransmitter to the receptor, the ion channels are opened allowing changes in ion distribution across the membrane which alters the potential. Since these ionotropic receptors are directly coupled to ion channels, the time course of the response to the neurotransmitter is in the range of μsec to msec (Taylor, 1990).

Neuromodulation is the alteration of a neuron's chemical and electrical properties in response to synaptic stimulation.
Neuromodulators are neuroactive compounds, such as peptides and indoleamines, which modulate synaptic responsiveness to excitatory and inhibitory neurotransmitters. Neuromodulators differ from neurotransmitters in that they can act both presynaptically and postsynaptically. Also, several neuromodulators (e.g. neuropeptides) are not enzymatically degraded. They simply diffuse out of the synaptic cleft. Finally, most neuromodulators induce their effects via activation of metabotropic receptors. Unlike ionotropic receptors, metabotropic receptors do not have as an integral part of the receptor an ion channel and thus must utilize other biochemical pathways. Metabotropic receptors are linked to second messenger systems which can alter the process of production, storage, release, reception, removal or uptake of a neurotransmitter. Since neuromodulators utilize second messenger systems, via the metabotropic receptor, the time course of these actions is much longer than that of the neurotransmitters (i.e. can last seconds to hours). Thus the classical excitatory and inhibitory effects of neurotransmitters on target neurons can be changed under various environmental conditions.

Neurotransmitters in the cerebellar nuclei.

The cerebellar nuclei have been shown to contain several different neuroactive compounds (i.e. amino acids, monoamines, and neuropeptides). At present the neurotransmitter utilized
by the olivary afferents is under debate. The candidate transmitters for this pathway are the excitatory amino acids aspartate and glutamate (Wiklund et. al., 1982; Zhang and Ottersen, 1993). The fibers that arise as collaterals or from nuclei that give rise to mossy fibers likely utilize glutamate (Stone, 1979; Somogyi et. al., 1986). The inhibitory inputs arising from Purkinje cells utilize gamma aminobutyric acid (GABA) (Ito et. al., 1964; 1970; Chan-Palay et. al., 1979). In addition to these classical neurotransmitters several other neuroactive substances have been detected within the cerebellar nuclei. These include taurine, acetylcholine, calcitonin gene-related peptide, and motilin (Oertel, 1993). Finally, the indoleamine serotonin has also been localized within a dense plexus of fibers that distribute throughout the cerebellar nuclei and cortex in the cat (Kerr and Bishop, 1991).

**Serotonin synthesis and activity.**

The indoleamine 5-hydroxytryptamine (serotonin; 5HT) was originally isolated from blood serum and was linked to the powerful contraction of smooth muscle (Erspamer, 1963; Feniuk, 1984). Since it’s purification, 5HT has been localized in many areas of the body including the brain. However, 5HT cannot cross the blood-brain barrier, thus the brain must synthesize this indoleamine from dietary tryptophan. The rate limiting step in the synthesis of serotonin is the conversion of tryptophan to 5-hydroxytryptophan via the enzyme tryptophan
hydroxylase. The enzyme amino acid decarboxylase converts 5-hydroxytryptophan to serotonin (Cooper et al., 1986). Even though brain serotonin accounts for only 1-2% of the total amount of serotonin in the body (Cooper et al., 1986), 5HT has been shown to play a role in neuronal differentiation and growth, neurotransmission, and neuromodulation (Lauder et al. 1978; Reader et al., 1979; Eaton and Salt, 1989; Strahlendorf et al., 1989; Lauder, 1990; Bishop and Kerr, 1992). In the cerebellar cortex, 5HT decreases the firing rate of spontaneous and excitatory amino acid activated Purkinje cells (Strahlendorf et al. 1989; Kerr and Bishop, 1992). 5-HT has also been shown to increase the firing rate of spontaneously active cerebellar nuclear neurons but depress the excitatory effects induced by the application of excitatory amino acids in vitro (Gardette et al. 1987; Cumming-Hood et al., 1990).

**Serotonin receptor subtypes.**

Over the past ten years, it has become increasingly evident that 5HT can utilize more than one receptor subtype, each with a unique pharmacology and signal transduction mechanism. Thus serotonin is capable of differential modulation of a neuron’s responsiveness to synaptic input depending upon which receptor subtype is present. Currently the 5HT receptor family is divided into the 5HT\textsubscript{1A}, 5HT\textsubscript{1B}, 5HT\textsubscript{1D}, 5HT\textsubscript{1E}, 5HT\textsubscript{1F}, 5HT\textsubscript{2A}, 5HT\textsubscript{2B}, 5HT\textsubscript{2C}, 5HT\textsubscript{3}, 5HT\textsubscript{4}, 5HT\textsubscript{5A,B}, 5HT\textsubscript{6}, and the 5HT\textsubscript{7} receptor subtypes (Table 1). The 5HT\textsubscript{1A} receptor has been shown, physiologically, to mediate some of
serotonin's effects in the rat and cat cerebellar corticies (Darrow et. al., 1990; Kerr and Bishop, 1992). Only recently have techniques progressed enough to anatomically determine which 5HT receptor subtype is present in the cerebellum. Several studies have shown the 5HT\textsubscript{1A} receptor is present within the molecular and Purkinje cell layers of the rat and human immature cerebellum (Daval et. al., 1987; Verge et.al., 1991; Matthiessen et. al., 1992; del Olmo et. al., 1994). One study, utilizing immunohistochemical techniques, demonstrated the 5HT\textsubscript{1A} receptor was localized on the Purkinje cell soma and dendrites in the immature rat cerebellum (Matthiessen et. al., 1993). However, only very small levels of this receptor subtype have been detected in the adult cerebellum. The 5HT\textsubscript{1B} receptor subtype has been localized within the Purkinje cell and molecular layers in the cortex and in the cerebellar nuclei (Maroteaux et. al. 1992; Saudou et. al., 1993; Palacios et. al., 1993; Neumaier et. al., 1993; Doucet et. al., 1993). However this receptor subtype may be specific to the mouse and rat (Hoyer et. al., 1985; Heuring et. al., 1986); it is absent in human, cow, pig, guinea pig, dog, chicken, and frog. The 5HT\textsubscript{2C} receptor subtype was also localized within the rat cerebellar nuclei (Palacios et. al., 1993), while the 5HT\textsubscript{1D} receptor was localized within the granule cell layer (Neumaire et. al., 1993). However, all of these studies utilized the rat and mouse models and as stated above, it was the 5HT\textsubscript{1A} receptor, not the 5HT\textsubscript{1B,2C, or 1D} receptors which was identified
in the adult cerebellar cortex of the cat (Kerr and Bishop, 1992). To date there have been no anatomical or physiological studies which have determined which 5HT receptor subtype is present in the adult cat’s cerebellar nuclei.

Objectives of the present study

This dissertation project has been divided into two parts. In Part I the origin of the 5HT projections to the cerebellar nuclei will be analyzed. The primary goal is to determine if these projections are collaterals of 5HT afferents that terminate in the cortex or whether they arise from distinct brainstem regions or neurons. Part II of this project will analyze the physiological effects of 5HT on neurons within the cerebellar nuclei and determine which 5HT receptor subtype is involved in mediating the effect.
Table 1. Serotonin Receptor Subtypes and Transduction Mechanisms

Table 1 illustrates the currently accepted classification of serotonin receptor subtypes along with their transduction mechanisms.
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<td>5HT\textsubscript{1A}</td>
<td>cAMP(−), K\textsuperscript{+} channel (+)</td>
<td>Peroutka, 1985; 1986; Fargin et. al., 1988</td>
</tr>
<tr>
<td>5HT\textsubscript{1B}</td>
<td>cAMP(−)</td>
<td>Hoyer et. al., 1985; Peroutka, 1985; Maroteaux et. al., 1992</td>
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<tr>
<td>5HT\textsubscript{1D}</td>
<td>cAMP(−)</td>
<td>Heuring and Peroutka, 1987; Branchek et. al., 1991</td>
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<tr>
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<td>cAMP(−)</td>
<td>McAllister et. al. 1992</td>
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<td>5HT\textsubscript{1F}</td>
<td>cAMP(−)</td>
<td>Adham et. al., 1993</td>
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<tr>
<td>5HT\textsubscript{2A}</td>
<td>IP3/DG(+)</td>
<td>Leysen, 1978; Julius et. al., 1990</td>
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<td>5HT\textsubscript{2B}</td>
<td>IP3/DG(+), Cl\textsuperscript{−} channel(+)</td>
<td>Schmuck et. al., 1994</td>
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<td>5HT\textsubscript{2C}</td>
<td>IP3/DG(+), Cl\textsuperscript{−} channel(+)</td>
<td>Pazos et. al., 1985; Yagaloff and Hartig, 1985; Julius et. al., 1988</td>
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<td>5HT\textsubscript{3}</td>
<td>Na\textsuperscript{+}/K\textsuperscript{+} internal channel(+)</td>
<td>Maricq et. al., 1991</td>
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<td>5HT\textsubscript{4}</td>
<td>cAMP(+)</td>
<td>Dumuis et. al., 1988</td>
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<td>unknown</td>
<td>Matthes et. al., 1992; Erlander et. al., 1993</td>
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<tr>
<td>5HT\textsubscript{6}</td>
<td>cAMP(+)</td>
<td>Monsma et. al. 1993; Ruat et. al., 1993</td>
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<tr>
<td>5HT\textsubscript{7}</td>
<td>cAMP(+)</td>
<td>Shen et. al., 1993</td>
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CHAPTER I

THE ORIGIN OF SEROTONINERGIC PROJECTIONS TO
THE CAT'S CEREBELLAR NUCLEI

INTRODUCTION

Previous immunohistochemical studies have localized serotoninergic (5HT) fibers within the cerebellar cortex of several species (Takeuchi et al. 1982a; Bishop and Ho, 1985; Bishop et al. 1985; Kerr and Bishop, 1991). These studies have shown that 5HT-like immunoreactivity is present in a dense plexus of beaded axons which are distinct from climbing and mossy fibers. Thus serotoninergic fibers represent a distinct class of afferents to the cerebellar cortex. Kerr and Bishop (1991), demonstrated that 5HT projections to the cat's cerebellar cortex originate from brainstem neurons located in the paramedian reticular formation, the lateral reticular nucleus, the lateral tegmental field, and the peri-olivary reticular formation; serotoninergic projections to the cerebellar cortex from raphe nuclei, postulated as sources of 5HT to the cerebellum by others (Shinnar et al. 1975; Chan-Palay, 1975), were not observed.

Serotonin positive varicosities have also been shown to
be uniformly distributed throughout all of the cat's cerebellar nuclei (Kerr and Bishop, 1991). It is not known, however, if these serotonergic projections arise from collaterals of fibers which distribute to the cortex, from a distinct population of serotonergic neurons within the brainstem or both. Thus the aim of this study was to determine the origin of serotonergic projections to the cerebellar nuclei and to determine if there is a differential source for each nucleus.
MATERIALS AND METHODS

Injection and Perfusion

Adult cats (n=15) were anesthetized by intraperitoneal injection of sodium pentobarbital (40mg/kg) and secured in a stereotaxic headholder. The occipital bone overlying the cerebellum was removed and latex microspheres (labeled with either Rhodamine or Fluorescein) were injected into the cerebellar nuclei through a Hamilton microsyringe (1μl). These injections were stereotaxically placed into either the medial, the interpositus, or the lateral cerebellar nucleus, according to coordinates from Berman’s (’68) atlas. The animals were allowed to survive for 24-36 hours, during which time the microspheres were retrogradely transported to brainstem neurons with axons at the injection site. The animals were then injected with pargyline (50mg/kg), one hour before sacrifice, followed by L-Tryptophan (50mg/kg), one half hour before sacrifice. The animals were anesthetized as described above and perfused through the aorta with 4% paraformaldehyde in phosphate buffered saline (pH 7.2). The brains were removed and immediately immersed in the same fixative for another 6 hours at 4°C followed by overnight immersion in Sorenson’s phosphate buffer (pH 7.2) containing 15% sucrose.
Tissue Processing

The cerebellum and brainstem were cut serially at 90 μm and 60 μm, respectively, on a freezing microtome. Transverse sections through the brainstem were collected and processed for immunohistochemistry. The tissue was placed into a primary antibody to 5HT (diluted 1:5000 with phosphate buffered saline plus 0.3% triton [PBT]) and incubated for 48-72 hours at 4°C with constant agitation. At the end of the incubation period the tissue was rinsed in PBS then placed into secondary antibody labeled with either rhodamine, in cases where animals received injections of fluorescein labeled microspheres, or fluorescein, when rhodamine labeled microspheres were injected (diluted 1:400 with PBT) and incubated for 2 hours at room temperature. The final incubation was followed by several rinses in PBS. The tissue was then mounted, briefly dipped in xylene and coverslipped with Krystalon (Diagnostic Systems Inc.). Sagittal sections of the cerebellum were also mounted for analysis of the injection sites.

Data Analysis

All sections were analyzed under a fluorescent microscope. Comparable results were obtained from either injections of rhodamine or fluorescein microspheres. Cells were identified as 1) 5HT-immunoreactive only; 2) retrogradely labeled only or 3) double-labeled. These three types of labeled neurons were plotted on a standardized series of
brainstem sections. The total number of double-labeled cells present in each brainstem source was determined for each nuclear injection. These data were then expressed as a percent of all double-labeled cells that projected to a particular nucleus. In addition, the number of retrogradely labeled cells within individual brainstem nuclei were counted and the percent that also contained 5HT-immunoreactivity was determined.
RESULTS

Distribution of serotonergic neurons

Figure 1 illustrates the distribution of 5HT neurons in the cat's brainstem. Serotonin immunoreactive neurons are located within the peri-olivary reticular formation (Fig. 1A,B), the lateral reticular nucleus (Fig. 1A,B), the lateral tegmental area (Fig. 1A,B), the dorsal tegmental nucleus (Fig. 1C-H), the locus coeruleus (Fig. 1C-H), and the raphe nuclei (Fig. 1A-H). These results are consistent with those of other studies which have looked at the distribution of this indoleamine in the cat (Takeuchi et al.1982b; Jacobs et al.1984; Kerr and Bishop, 1991).

Distribution of retrogradely-labeled neurons

Figures 2-4 illustrate the distribution of retrogradely labeled and double-labeled cells following injections into different cerebellar nuclei. In this analysis 5 adjacent sections are represented by each drawing. Due to the large number of retrogradely-labeled cells, each diagram illustrates their distribution within a single tissue section. However, due to the lower number of double-labeled cells, each diagrammed section represents the distribution of double-labeled cells from all adjacent tissue sections. Neurons containing retrogradely transported microspheres following
injections of the medial nucleus are located in the locus coeruleus (Fig. 2C-H), the dorsal raphe nucleus (medial division) (Fig. 2E-H), the superior central nucleus (Fig. 2D-H), the inferior central nucleus (Fig. 2B,C), the lateral tegmental field (Fig. 2B), the external cuneate nucleus (Fig. 2B), the lateral reticular nucleus (Fig. 2B), the dorsal tegmental field (Fig. 2C-H), the tegmental reticular nucleus (Fig. 2D,E,G), the pontine grey (Fig 2D,G), the peri-olivary region (Fig. 2B), and the inferior olive (Fig. 2B). The olivary projections arise from the caudal medial accessory olive, with few to none from the dorsal accessory or the principle nuclear portions of the olivary complex.

Injections into the anterior and posterior interpositus nucleus produced retrograde labeling in the same nuclei that were labeled after injections of the medial nucleus (Fig. 3B-G). Injection of the interposed nuclei also labeled neurons within the medial and dorsal accessory nucleus of the olive but few to none within the principle olivary nucleus (not illustrated).

Injections of the lateral nucleus, like those within the medial and interposed nuclei, labeled neurons within the medial division of the dorsal raphe nucleus (Fig. 4D-G) the locus coeruleus (Fig. 4B-G), the superior central nucleus (Fig. 4C-G), the inferior central nucleus (Fig. 4B), the dorsal tegmental field (Fig. 4C-G), and the basilar pons (Fig. 4C-G). In addition, the injections labeled neurons within the
peri-olivary region, the reticular tegmental nucleus (Fig. 4C-G), and the area surrounding the dorsal motor nucleus of the vagus. The lateral nucleus injections also labeled neurons within the medial accessory and principle nuclei of the inferior olive but not within the dorsal accessory nucleus.

**Distribution of double-labeled neurons**

Figure 5 illustrates examples of double-labeled cells in the locus coeruleus following injections of the lateral and medial nuclei, respectively. Following injections of the medial nucleus, neurons containing both retrogradely transported microspheres and serotonergic-like immunoreactivity were located bilaterally in the medial division of the dorsal nucleus of the raphe (Fig. 2D-H), the locus coeruleus (Figs. 2C-H; 5C,D), the pericentral division of the dorsal tegmental nucleus (Fig. 2C-H), the central superior nucleus (Fig. 2D), the central inferior nucleus (Fig. 2B,C), and the peri-olivary reticular formation (Fig. 2B). The first three of these nuclei contained the greatest number of double-labeled neurons.

Of the total number of double-labeled neurons observed in 3 cases, 42% (n=40 out of 96) were located within the dorsal raphe. The locus coeruleus and the dorsal tegmental nucleus contained, 29% (28/96) and 17% (16/96) of the double-labeled neurons, respectively. The remaining double-labeled neurons were located within the central superior nucleus, the central inferior nucleus and the peri-olivary reticular formation, 2%
(2/96), 7% (7/96) and 3% (3/96), respectively (Figure 6A). The number of double-labeled neurons in each of the above mentioned nuclei represents only a percentage of the total number of retrogradely labeled neurons observed. Of the total number of retrogradely labeled neurons located within the dorsal raphe, 33% (40/120) were also 5HT immunoreactive. In the locus coeruleus and the dorsal tegmental nucleus, 20% (28/124) and 20% (16/78), respectively, of the retrogradely labeled cells were double-labeled. In the central superior nucleus, the central inferior nucleus, and the peri-olivary reticular formation, 20% (2/10), 19% (7/37) and 20% (2/10), respectively, of the retrogradely labeled cells were also immunolabeled (Figure 6B).

After injections of the interpositus nucleus, 63% (80/127) of the double-labeled neurons were found in the dorsal raphe nucleus (Fig. 3D-G). The locus coeruleus (Fig. 3C-G) and the dorsal tegmental nucleus (Fig. 3D-G) contained, 21% (27/127) and 14% (18/127), respectively, of the double-labeled neurons. 2% (3/127) of the double-labeled neurons were located within the central inferior nucleus (Fig. 3B). Of the total number of retrogradely labeled neurons located within the dorsal raphe, 25% (80/310) were also serotonin immunoreactive. In the locus coeruleus, the dorsal tegmental nucleus, and the central inferior nucleus, 8.1% (27/332), 9.1% (18/197), and 7% (3/44), respectively, of the retrogradely labeled neurons were also immunolabeled (Figure 6B).
In cases where the lateral nucleus was injected, 43% (31/72) of the double-labeled neurons were located in the dorsal raphe nucleus (Fig. 4C-G). The locus coeruleus (Fig. 4B-G; 5A,B), the dorsal tegmental nucleus (Fig. 4D-G), and the central superior nucleus (Fig. 4C-G) contained, 25% (18/72), 25% (18/72), and 7% (5/72), respectively, of the total number of double-labeled neurons observed (Figure 6A). Fourteen percent (31/224) of the neurons within the dorsal raphe were double-labeled following injections of the lateral nucleus. Double-labeled neurons within the dorsal tegmental field, locus coeruleus, and the superior central nucleus represented 12% (18/145), 16% (18/114), and 7% (5/71), respectively, of the retrogradely labeled neurons (Figure 6B).
DISCUSSION

There are several problems inherent in double label paradigms. The first involves the interpretation of negative data. It is possible that not all serotonergic cells are immunolabeled. The present study utilized pargyline, a monoamine oxidase inhibitor, and L-tryptophan, a serotonin precursor, to insure that as many 5HT positive cells as possible were labeled. Further, only those axons located within the injection site will be retrogradely labeled. Thus, we likely have not retrogradely labeled all neurons that project to a particular cerebellar nucleus, as the fluorescent microspheres have limited spread. Therefore, it is probable that the data represents an underestimation of the actual number of 5HT cells that project to a given cerebellar nucleus. Two other problems that must be considered are spread of the retrograde marker away from the injection site as well as injury to fibers of passage. In the present study we observed minimal to no spread of the microspheres into the cortex following injections of the cerebellar nuclei. The problem of injury to fibers of passage is obviated to some extent by the fact that mossy fiber and climbing fiber afferents supplying the cerebellar cortex course around, not through the cerebellar nuclei (Dietrichs, 1983a; Dietrichs et
al, 1983b). However, we cannot rule out the possibility that some fibers enter the nuclei on the way to the cortex. Katz (1984) demonstrated that fibers take up the microspheres only if they are cut. Since the cerebellar nuclei lay deep within the cerebellum, some fibers that project to the cortex may have been lesioned as the microsyringe was lowered. In order to avoid additional lesioning due to hydraulic pressure, injections were made slowly. Injured fibers of passage are not likely a major problem as each of the brainstem nuclei which contained retrogradely labeled neurons have previously been shown to project to the cerebellar nuclei (Somana and Walberg, 1978; Dietrichs and Walberg, 1987). Further, the present study has shown that the major source of serotoninergic projections to the cat’s cerebellar nuclei are brainstem regions which are rostral to those areas of the brainstem that project to the cerebellar cortex.

The cortex receives serotoninergic projections from the pontine reticular formation, the lateral reticular nucleus, the lateral tegmental field, and the peri-olivary reticular formation (Kerr and Bishop, 1991). Several studies have suggested that there are two distinct patterns of termination for afferent systems to the cerebellum (Dietrichs, 1983a; Dietrichs and Walberg, 1987; Qvist, 1989). The findings of these studies indicate that afferents either terminate in both the cerebellar cortex and the cerebellar nuclei or only in the cerebellar cortex. The data from the present study represents
a third pattern in which a chemically defined system of afferents projects only to the cerebellar nuclei. It should be noted that the medial cerebellar nucleus is the only one which receives a few serotoninergic projections from a brainstem region which also provides 5HT-immunoreactive afferents to the cortex, specifically the peri-olivary reticular formation. It should be emphasized, however, that the medial nucleus receives most of its serotoninergic projections from more rostral brainstem regions.

It has been demonstrated previously that the dorsal tegmental nucleus and the locus coeruleus project to the cerebellum (Eller and Chan-Palay, 1976; Somana and Walberg, 1978; Dietrichs, 1985). Somana and Walberg (1978) demonstrated a few retrogradely labeled neurons within the locus coeruleus following injections of HRP into the cat's medial and interposed nuclei, but not after injections of the lateral nucleus. In contrast, Eller and Chan-Palay (1976) demonstrated a few retrogradely labeled neurons within these two brainstem nuclei following injections of HRP into the rat's lateral cerebellar nucleus. The present study has shown that the locus coeruleus has substantial projections to all of the cerebellar nuclei in the cat. These discrepancies may reflect limitations of the different techniques utilized by each study or species differences. Other studies have demonstrated that the dorsal tegmental nucleus and the locus coeruleus contain serotoninergic neurons (Takeuchi et
However, this is the first study to provide experimental evidence of serotoninergic projections to the cerebellar nuclei which arise from these brainstem regions. The present study has also demonstrated that the number of double-labeled neurons within each brainstem nucleus represents only a small percentage of the total number of retrogradely labeled cells. Thus, the cerebellar nuclear afferents from a particular brainstem nucleus are not homogeneous. Some are serotoninergic but most are non-serotonergic.

Several studies have reported that few if any serotoninergic axons in the cerebellar cortex of the rat, opossum or cat arise within the raphe nuclei (Bishop and Ho, 1985; Walker et al. 1988; Kerr and Bishop, 1991). In the present study, however, we have presented evidence for serotoninergic projections to the cerebellar nuclei which originate within rostral raphe nuclei. In fact, the dorsal raphe was one of the major sources of serotoninergic projections.

The cerebellar cortex and nuclei receive excitatory input from neurons located within various brainstem nuclei involved in control of motor activity (lateral reticular nucleus, inferior olivary complex, reticular formation). In addition, superimposed on these inputs are serotoninergic projections from diverse brainstem regions. The 5HT input to the cortex is derived from nuclei that receive input primarily from the
spinal cord and cerebral cortex. These inputs likely relay information related to the status of an ongoing movement. In contrast the 5HT afferents to the cerebellar nuclei are derived from brainstem centers that are not directly involved in relaying information about motor activity. The dorsal raphe nucleus and superior central nucleus both receive their primary input from limbic regions as well as the superior vestibular nucleus, the substantia nigra, and the ventral tegmental areas (Kalen et al.1985). The locus coeruleus has diffuse projections to spinal, thalamic, cerebellar and forebrain levels and appears to be involved in arousal and autonomic functions (Bolme et al.1972; Jones et al.1973; Cedarbaum and Aghajanian, 1978). Neurons in the locus coeruleus are thought to play a role in relaying information about the general attention state of the animal to various regions of the CNS. The present study has shown that both the dorsal raphe and locus coeruleus are major sources of 5HT afferents to each of the cerebellar nuclei. At present it is not known how serotonergic neurons are activated or under what circumstances 5HT is released in the cerebellum. However, since the cerebellar cortex and nuclei receive 5HT afferents from brainstem nuclei that are functionally distinct, the activity of the two cerebellar regions may be modulated by this indoleamine in response to unique inputs.

With the exception of input from the inferior olive and a few other brainstem nuclei it is becoming evident that the
cerebellar cortex and nuclei receive input from distinct populations of brainstem neurons. These differences in afferent input, both serotoninergic and non-serotoninergic, suggest that the cerebellar cortex and nuclei do not process all information in parallel. Rather each responds to and integrates distinct inputs which ultimately result in a precise co-ordination of motor activity.
Figure 1. Distribution of Serotoninergic Neurons within the Cat’s Brainstem

Drawings of transverse sections through the cat’s brainstem that illustrate the distribution of serotoninergic neurons (closed stars) plotted on standardized sections. The calibration bar = 3.5mm and applies to Figures 2-4.
FIGURE 1
Figure 2. Distribution of Serotonergic Neurons that Project to the Cerebellar Medial Nucleus

Figure 2A illustrates a representative injection site in the medial nucleus of the cerebellum. The solid area represents the primary injection site and the cross-hatched area indicates light spread of the microspheres. Figs. 2B–H illustrate the distribution of retrogradely labeled neurons (dots) and double-labeled neurons (stars) in the brainstem plotted on standardized sections.
FIGURE 2
Figure 3. Distribution of Serotonergic Neurons that Project to the Cerebellar Interposed Nuclei

Figure 3A Illustrates a representative injection site in the interposed nuclei of the cerebellum. The solid area represents the primary injection site and the cross-hatched area represents the spread of the beads. Figs. 3B-G illustrate the distribution of retrogradely labeled neurons (dots) and double-labeled neurons (stars) in the brainstem plotted on a standardized series of sections. No double-labeled cells were found in the medulla.
FIGURE 3
Figure 4. Distribution of Serotonergic Neurons that Project to the Cerebellar Lateral Nucleus

Figure 4A illustrates a representative injection site in the lateral nucleus of the cerebellum. The solid area represents the primary injection site. Figs. 4B–G illustrate the distribution of retrogradely labeled neurons (dots) and double-labeled neurons (stars) in the brainstem, plotted on a standard series of sections. No double-labeled cells were found in the medulla.
FIGURE 4
Figure 5. Double-labeled Neurons within the Central Grey Area and Locus Coeruleus

Photomicrographs of double-labeled neurons in the central grey and the locus coeruleus. Figures 5A and B illustrate a neuron in the central grey that is immunoreactive for 5HT (5A) and also retrogradely labeled with fluorescent microspheres (5B) following an injection into the lateral cerebellar nucleus. Figs. 5C & 5D illustrate a double-labeled cell in the locus coeruleus following an injection into the medial nucleus. The calibration bar = 40μm in 5A-B, 30μm in 5C-D.
FIGURE 5
Figure 6. Percent of Retrogradely and Double-Labeled Neurons

Fig. 6A histogram illustrating the percentage of all double-labeled cells arising from specific brainstem nuclei. The projection to each cerebellar nucleus is indicated. Fig. 6B histogram illustrating the percentage of neurons within a specific brainstem nuclei that are both serotoninergic and which also project to each of the cerebellar nuclei.
A Percent of all double-labeled neurons

B Percent of retrogradely labeled neurons

FIGURE 6
CHAPTER I REFERENCES


CHAPTER II

THE PHYSIOLOGICAL EFFECTS OF SEROTONIN
ON NEURONS IN THE CAT'S CEREBELLAR NUCLEI

INTRODUCTION

It is well established that cerebellar efferents originate from neurons located within the cerebellar nuclei (Ito, 1984) and that the activity of these neurons is regulated by excitatory inputs derived from the brainstem (Eccles, 1974; Kitai et. al., 1977; McCrea et. al., 1977). Some of these inputs arise as collaterals of axons that terminate as climbing fibers and mossy fibers in the cerebellar cortex (Chan-Palay, 1973; Qvist, 1989; Dietrichs and Walberg, 1987) and contain aspartate or glutamate (Stone, 1979; Wiklund et. al., 1982; Somogyi et. al., 1986). Other inputs arise from brainstem nuclei that project only to the cerebellar nuclei (Kitzman and Bishop, 1994) and contain serotonin. Cerebellar nuclear neurons also receive inhibitory inputs which arise from Purkinje cells in the cerebellar cortex and contain GABA (Ito et. al., 1964; 1970; Wojtowicz et. al., 1978; Chan-Palay, 1979; Aoki et. al., 1989). In addition to these classically defined afferent systems a third
chemically defined system, containing monoamines such as serotonin, has been shown to form a beaded plexus within the deep cerebellar nuclei of several species of mammals (Takeuchi et. al., 1982; Bishop and Ho, 1985; Bishop et. al., 1985; Walker et. al., 1988; Kerr and Bishop, 1991, Kitzman and Bishop, 1994). In the cat, serotonin is uniformly distributed within all four cerebellar nuclei (Kerr and Bishop, 1991) as well as the cerebellar cortex. The origins of the serotonergic afferents to each of the four cerebellar nuclei arise primarily from the dorsal raphe nucleus, the nucleus locus coeruleus, the dorsal tegmental nucleus (Kitzman and Bishop, 1994). These brainstem regions are distinct from those that give rise to 5HT projections to the cerebellar cortex (Kerr and Bishop, 1991).

Several studies have demonstrated a modulatory effect of 5HT on the activity of cerebellar cortical neurons (Strahlendorf et. al., 1984; Armstrong and Hay, 1987; Strahlendorf et. al., 1989; Kerr and Bishop, 1992). However, there have been few studies that have looked at the response of cerebellar nuclear neurons to the application of 5HT. Studies conducted in slice preparations indicate that 5HT modulates both spontaneous and excitatory amino acid induced activity of neurons within the cerebellar nuclei (Gardette et. al., 1987; Cumming-Hood et. al., 1990). However these slices were from young rats (age 17-25 days); thus these neurons may not have developed mature physiological properties. There
have been no studies that have determined the physiological effects of 5HT on the activity of cerebellar nuclear neurons in vivo. Thus the purpose of the present study is to determine, in vivo, if 5HT alters the activity of neurons in the nucleus interpositus of the adult cat. Specifically, the interactions between 5HT and synaptically driven activity induced by stimulation of the inferior olivary complex (IOC) were analyzed as were interactions between 5HT and glutamate, aspartate, quisqualate, NMDA, and GABA.

Serotonin exerts its effects on neuronal activity through several distinct receptor subtypes. The 5HT$_{1A}$ subtype has been shown to mediate serotonin's modulatory effects within the cerebellar cortex and cerebellar nuclei of the cat and rat, respectively (Kerr and Bishop, 1992; Cumming-Hood et. al., 1990) while the 5HT$_{1B}$ and 1C receptor subtypes have been anatomically localized in the rat cerebellar nuclei (Boschert et. al., 1993; Palacious et. al., 1993). However, there have been no studies, anatomical or physiological, that have looked at which subtype is present within the cat's cerebellar nuclei. Thus, another goal of this study is to physiologically demonstrate which 5HT receptor subtype(s) mediate the effects of 5HT within the cerebellar nuclei. Portions of this study have been published in abstract form (Kitzman and Bishop, 1993).
Adult cats (n=29) of both sexes were anesthetized with a combination of either sodium thiamylal (Surital 40 mg/kg) or Pentothal (55 mg/kg) and α-chloralose (70 mg/kg). The trachea and the cephalic vein were both cannulated and the animals were secured in a stereotaxic frame. Bipolar electrodes were stereotaxically (Berman, 1968) placed within the right inferior olivary complex (IOC). The bone and the dura covering lobules V and VI was removed and recording electrodes were stereotaxically lowered into the left interposed nuclei. The animals temperature was monitored and maintained at 37°C for the entire experiment. The animals reflexes were tested and supplementary doses of the anesthetic were administered when needed.

Five-barrel micropipettes were pulled on a Narisinghe vertical pipette puller and the tips broken, under microscopic control, to a final diameter of 2-4μm. The center barrel was filled with 4 M NaCl for the purpose of recording neuronal activity. A second barrel was also filled with 4 M NaCl and functioned as an automatic balancing circuit that eliminated the possibility that the observed responses were caused by the applied current and not the substance being tested. The remaining side-barrels were filled with combinations of the following substances: 0.05 M 5-hydroxy-tryptamine creatine sulfate (5HT, serotonin), pH 4.5; 0.2 M glutamate, pH 7.5; 0.2
M aspartate, pH 7.5; 0.2 M GABA, pH 4.3; 0.2M N-methyl-D-aspartate, pH 7.5; 5.0mM Quisqualate pH 7.5; 0.02 M (±)-8-hydroxy-2-(di-n-propylamino)tetralin hydrobromide (8-OH-DPAT) a 5HT1A agonist, pH 4.5; 0.2 M 2-(4-(4-(2-pyrimidinyl)-1-piperazinyl)butyl-1,2-benzisothiazol-3-(2H)one-1,1-dioxidehydrochloride (ipsapirone), a 5HT1A agonist, pH 4.5 (given to the project by Miles Laboratory). All solutions were freshly prepared or stored at -20°C.

During advancement of the electrode, appropriate retaining currents of at least 15-20 nA were applied to each drug barrel to prevent leakage of the various chemicals. Following extracellular isolation of a unit the currents were reversed to eject the substances. Quisqualate, glutamate, aspartate, and NMDA were ejected with anodal current, all other drugs were applied with cathodal current. The extracellular responses were amplified on a Dagan preamplifier, monitored on an oscilloscope and converted to a uniform voltage pulse by passage through a window discriminator. These pulses were counted on a ratemeter over 1 sec. intervals and displayed on a strip chart recorder. At the conclusion of the experiment the anesthetized animal was perfused through the aorta with saline followed by 3.5% paraformaldehyde. The brain was removed and 90 µm sections were cut an a freezing microtome, mounted on glass slides, counterstained, and cleared in xylene in order to analyze the placement of the electrodes.
Data analysis

As stated above, changes in extracellular responses to the iontophoretic application of excitatory amino acids (glutamate, aspartate, quisqualate, and NMDA), 5HT and GABA were displayed on a strip chart recorder. The chart recordings were analyzed to determine the change in neuronal firing rate caused by the interaction between excitatory amino acids and 5HT; between the excitatory amino acids and GABA; and between GABA and 5HT.

In addition to interactions between 5HT and excitatory amino acids, we also determined the effects of 5HT on synaptic activity mediated by stimulation of the inferior olivary complex (IOC). Extracellular spike potentials were photographed directly from the oscilloscope. The photographs of the oscilloscope traces were measured for: 1) the change in the number of spikes, spike shape and amplitude during spontaneous activity versus IOC stimulation only or IOC stimulation plus concomitant application of 5HT; 2) changes in the interval between the shock artifact and the first (initial) spike before and during 5HT application (treatment); 3) changes in the interspike interval between the initial spike and subsequent spikes before and during 5HT application (treatment). Measurements were made from three consecutive traces and the average latencies were recorded. Differences in initial spike latency between IOC stimulation only and IOC stimulation plus 5HT application were evaluated using a One-
Way Analysis of Variance (ANOVA) with repeated measures for treatment and trials. Treatment refers to application of 5HT and trial indicates the number of cells/cat that were recorded.
RESULTS

Three criteria were used to confirm that the recorded units were located within the cerebellar nuclei: 1) the distance from the cerebellar surface to the unit was measured and compared to the stained sections of the cerebellum containing the electrode tracts; 2) during stimulation of IOC the recorded units demonstrated an initial action potential, followed by an interval with no responses, followed by additional action potentials; 3) physiologically there were no complex spikes recorded during IOC stimulation (which would have indicated that the recorded unit was a Purkinje cell).

The effect of serotonin on spontaneous activity.

Of the 119 nuclear neurons isolated in this study, 28 were spontaneously active. Iontophoretic application of serotonin suppresses spontaneous activity in 72% (n=20) of the units tested in a dose-dependent manner. An example of a spontaneously active unit is shown in Fig. 7A. This cell had an average firing rate of 40 spikes/sec. Simultaneous application of 5HT decreased the spontaneous firing rate to 10 spikes/sec which represents a 75% reduction in activity (Fig. 7B). When the current to the 5HT barrel is reversed to retain the indoleamine, the unit recovers to the pre-serotonin firing rate (Fig. 7C). The time course and dose-dependency of the
effects are shown in Fig. 7D. At 50nA, 5HT suppresses firing rate 34%; as the current was increased the degree of suppression is also enhanced. For example at 70nA and 90nA, 5HT suppresses firing rate 53% and 70%, respectively. At 118nA a complete suppression of activity was achieved. Two of the 28 units demonstrated an increase in firing rate. Following the application of 5HT for 5 sec one neuron had increased it's firing rate from 33 spikes/sec to 55 spikes/sec. After recording from the neuron for 13 min the application of 5HT increased the firing rate from 33 spikes/sec to only 34 spikes/sec. The second neuron initially demonstrated an increase in firing rate from 55 spikes/sec to 67 spikes/sec. After a period of 14 min the application of 5HT induced a decrease in the firing rate from 55 spikes/sec to 11 spikes/sec. In six of the 28 units, serotonin had no effect on spontaneous activity. Three of the tested units that were suppressed by 5HT, demonstrated a partial recovery of firing activity during the application of 5HT.

The effect of 5HT on synaptic activity.

Bipolar electrodes were placed in the inferior olivary complex on the side contralateral to the recording site in the cerebellar nuclei and the interaction between 5HT and IOC stimulation on neuronal activity was determined. Stimulation of IOC induced a characteristic response in all of the recorded units (n=12); which includes an initial excitatory response followed by a period of inhibition, followed by
additional action potentials. The iontophoretic application of 5HT has no effect on the initial spike but decreases the number of action potentials in the second excitatory phase. Although it did not block the initial spike potential it increased the initial latency 36% (8msec) from stimulus artifact to response. Prior to 5HT application the average latency for the 12 cells was 14.7 msec (± 2.9). During application of 5HT the latency increased to 22.7 msec (± 2.9). This difference is significant based on ANOVA analysis (p<0.07). The application of 5HT also increased the period of suppression resulting in a greater interspike latency between the initial and later spikes by 24% (10msec), in eight of the recorded neurons. Prior to 5HT application the average interspike interval for the eight cells was 32.7 msec (± 2.8). During application of 5HT the interval increased to 42 msec (± 2.8). This difference is significant at p<0.05. Figure 8A demonstrates a spontaneously active cell. Stimulation of IOC induces an initial spike, followed by a period of no activity, followed by additional spikes (Fig. 8B). Concomitant application of 5HT increases the interval between the initial spike and later spikes (interspike interval) (Fig. 8C). In Fig. 8D the cell return to a pre-serotonin pattern of activity. The remaining four neurons demonstrated a complete suppression of neuronal activity after the initial spike. Figure 8E demonstrates a unit that was not spontaneously active. Stimulation of IOC (-50) induces an initial spike
followed by a period of inhibition followed by a second spike (Fig. 8F). Concomitant application of 5HT (30nA) causes an increase in the latency between the shock artifact and the initial spike. There was also a suppression of activity following the initial spike (Fig. 8G). In Fig. 8H the cell recovers to a pre-serotonin pattern of activity. The application of 5HT did not change the spike amplitude.

The histogram shown in Fig. 9 summarizes the effects of 5HT on the initial and interspike intervals induced by the stimulation of the IOC.

The effect of serotonin on excitatory amino acid induced activity.

The iontophoretic application of aspartate augments the firing rate in all units tested (n=16). Concomitant application of 5HT induces a partial suppression of the augmenting action of aspartate in 100% of the units tested. The unit in Fig. 10A demonstrates low spontaneous activity. The iontophoretic application of aspartate increases the firing rate 7 fold (Fig. 10B). The concomitant application of 5HT decreases the aspartate induced firing rate 50% (Fig. 10C). In Fig. 4D, the cell recovers to pre-serotonin firing rate following the discontinuation of 5HT application. Figure 10E is a chart recording that demonstrates the time course and dose dependency of this effect.

Ten units demonstrated an increase in firing rate following the application of glutamate. Concomitant
application of 5HT partially suppresses the augmenting action of glutamate in 90% of the units tested. One unit was non-responsive to 5HT. Figure 10F is a chart recording that demonstrates the time course and dose dependency of this effect. At 10nA firing rate decreases 17%; while at 20nA firing rate decreases 75%, and at 30nA firing rate decreases 88%.

Forty five units demonstrated an increase in firing rate following the application of quisqualic acid. Simultaneous application of serotonin suppresses the activity of 78% of the quisqualate augmented units (n=35); the remaining quisqualate activated units demonstrated no response to the application of 5HT. The suppressive effect of 5HT was dose dependent. The unit in Fig. 11A is spontaneously active. The iontophoretic application of quisqualate increased firing rate over 3 fold (Fig. 11B). Concomitant application of 5HT blocks the augmenting action of quisqualate 30% (Fig. 11C). In Fig. 11D, the cell recovers to pre-serotonin firing rate following the discontinuation of 5HT application. The dose dependent manner of 5HT’s suppression of quisqualate augmented firing rate is demonstrated in Fig. 11E. At 2nA there was no change in firing rate; while at 10nA, 20nA and 40nA firing rate decreased 50%, 63%, and 92%, respectively.

The iontophoretic application of NMDA augmented the firing rate in twenty units. Concomitant application of 5HT suppresses the activity of 85% of the NMDA augmented units
(n=17) in a dose dependent manner, while having no effect on the remaining three units. The unit in Fig. 12A is spontaneously active. The iontophoretic application of NMDA increased firing rate over 3 fold (Fig. 12B). Simultaneous application of 5HT suppresses the augmenting action of NMDA by 29% (Fig. 12C). Following the application of 5HT the unit recovers to pre-serotonin firing rate (Fig. 12D). Figure 12E demonstrates the suppressive effect of 5HT on NMDA augmented firing rate is dose dependent.

The histogram in Fig. 13 summarizes the effects of 5HT on excitatory amino acid augmented activity.

To determine whether serotonin differentially effects neurons that are activated by NMDA receptors vs non-NMDA receptors, nine units were alternately activated with NMDA and quisqualate. 5HT had a greater suppressive effect on quisqualate-induced activity as compared to that evoked by NMDA (Figs. 14A & B). Figure 14A shows a unit which is alternately activated with NMDA and quisqualate. Concomitant application of 5HT at 100nA suppresses NMDA augmented firing rate 26% compared with 100% suppression of quisqualate-activated firing rate. Figure 14B summarizes the differences in 5HT suppression on NMDA-activated firing rate verses quisqualate-activated firing rate for nine different units (C1-C9). In all cases quisqualate-augmented activity was suppressed more than NMDA-augmented activity.
Interaction between 5HT and GABA

Along with excitatory inputs from the brainstem, the cerebellar nuclei also receive inhibitory GABAergic projections from the cerebellar cortex (Ito et al., 1964; 1970; Chan-Palay et al., 1979). Thus interactions between serotonin and GABA on cerebellar nuclear neuronal firing rate were tested. Iontophoresis of GABA suppresses the firing rate of all spontaneous and glutamate, aspartate, quisqualate, and NMDA activated units (n=23). Figure 15A demonstrates the suppressive effect of GABA on a spontaneously active neuron. At 20nA firing rate decreases 50%; while at 30nA and 60nA GABA suppress the units firing rate 62% and 100%, respectively. This unit becomes more responsive to the application of GABA over time. Initially at 40nA firing rate decreases 22%. However, later in the trial the application of GABA at 40nA causes a 100% decrease in firing rate. In 50% of the spontaneously active units concomitant application of 5HT to units pre-conditioned to GABA causes a potentiation of GABA’s suppressive effect (a change in neuronal firing rate that is greater than the sum of the individual responses induced by either 5HT or GABA), while in the remaining units concomitant application of 5HT had an additive effect on GABA suppression (a change in neuronal firing rate that is equal to the sum of the individual responses induced by either 5HT or GABA). However, concomitant application of GABA to a unit pre-conditioned to 5HT enhances the 5HT suppression in an additive
manner. In a few neurons the application of GABA to a unit pre-conditioned to 5HT has minimal or no effect on 5HT suppression (Fig. 15B). 5HT and GABA were applied at submaximal current. In Fig. 15B the application of either GABA at 5nA or 5HT at 118nA had minimal effects on the units firing rate. Pre-conditioning the neuron with GABA (5nA) had no effect on the firing rate. Concomitant application of 5HT (118nA) still had no effect on the neuronal firing rate. However, as soon as the application of 5HT is terminated, the firing rate decreased 80% and this suppression continued as long as GABA was applied. In contrast, the application of GABA to a unit pre-conditioned to 5HT had no effect on firing rate.

Serotonin potentiated the suppressive effect of GABA in 78% and 60% of the units augmented by quisqualate and aspartate, respectively; while having an additive effect on the remaining units. GABA potentiated the suppressive effect of 5HT in 50% of the quisqualate-activated units while having an additive effect on the remaining units. The suppressive effect of 5HT on aspartate activated neurons was enhanced by GABA in an additive manner. In Fig. 15C 5HT and GABA suppress firing rate in an additive manner. Simultaneous application of 5HT and GABA suppresses quisqualate-induced firing rate 45%. At 10nA GABA decreases the firing rate 34%. Concomitant application of 5HT suppresses the firing rate an additional 33% (a total suppression of 67%). Conditioning the cell to 5HT decreases the firing rate 17%. Concomitant
application of GABA suppresses the firing rate an additional 55\% (a total suppression of 62\%). In Fig. 15D the application of 5HT at 80nA suppresses firing rate 28\% while GABA at 15nA suppresses firing rate 34\%. The application of GABA during conditioning with 5HT suppresses quisqualate-induced firing rate 84\%. When GABA application is discontinued the firing rate returns to pre-GABA levels. The application of 5HT during conditioning with GABA also suppresses firing rate 78\%. However, this potentiation of GABA’s suppression continues even when the application of 5HT is discontinued. Serotonin potentiated the suppressive effect of GABA in two units augmented by NMDA; while having an additive effect on a third unit.

**Determination of serotonin receptor subtype**

Since it has been shown that the 5HT\textsubscript{1A} receptor is functional in the cat’s cerebellar cortex (Kerr and Bishop, 1992), this receptor subtype was tested for. Eight neurons demonstrated a suppression of neuronal firing rate following the application of ipsapirone, a 5HT\textsubscript{1A} receptor agonist. Figure 16A demonstrates that ipsapirone, like 5HT, suppresses glutamate augmented activity in a dose dependent manner. At 40nA firing rate decreased 50\%; while at 50nA firing rate decreased 67\%. The percent decrease in firing rate was similar to that observed following the application of 5HT at the same current level (50\% suppression by Ipsapirone verses 82\% suppression by 5HT at 40nA and 92\% decrease by ipsapirone
verses 100% decrease by 5HT at 60nA). One neuron showed no response to ipsapirone. However, this same neuron was also non-responsive to the application of 5HT.

The application of 8-OH-DPAT, another 5HT$_{1A}$ agonist, suppressed firing rate in all neurons tested (n=5). Figure 16B shows the suppressive effect of 8-OH-DPAT, on a neuron activated by quisqualate, is dose dependent. At 20nA firing rate decreased 45%; while at 40nA firing rate decreased 90%. One neuron showed no response to 8-OH-DPAT. However, this same neuron was also non-responsive to the application of 5HT.
DISCUSSION

The present study is the first to demonstrate the effects of 5HT on cerebellar nuclear neurons in vivo. The data presented indicate that 5HT has a suppressive effect on spontaneous firing rate. These findings differ with those in previous studies conducted in vitro (Gardette et. al., 1987; Gardette and Crepel, 1993; Cumming-Hood et. al., 1990). Gardette et. al. (1987; 1993) demonstrated that 5HT increased the firing rate of spontaneously active neurons; while Cumming-Hood et. al. (1990) observed both excitation and suppression of spontaneous nuclear activity in response to the application of 5HT. There are several possible reasons for the observed differences in neuronal response to the application of 5HT. First, the in vitro experiments utilized rat cerebellar slice preparations in which the extrinsic input to the cerebellar nuclei had been removed. The removal of these inputs could alter the physiological responsiveness of the neurons. Secondly, distinct 5HT receptor subtypes are present in the rat that have not been demonstrated in the cat (discussed below). Thus 5HT could have differing effects depending upon which receptor subtype is present.

The present study is in agreement with previous studies with respect to the interaction between 5HT and excitatory
amino acids on the activity of cerebellar nuclear neurons. Uniformly, 5HT suppresses neuronal activity that has been augmented by the application of aspartate and glutamate, the putative excitatory transmitters of climbing and mossy fibers, respectively (Stone, 1979; Wiklund et. al., 1982; Somogyi et. al., 1986). Also, 5HT has a greater suppressive effect on cells activated by quisqualate as compared with NMDA-activated neurons.

The quisqualate/AMPA receptor is a Na⁺/K⁺ channel that has been proposed to be involved in fast depolarization of the neuron. The currents induced by the activation of this receptor have a short induction time and a short duration. The NMDA receptor-channel complex differs from the quisqualate/AMPA receptor in that it is neurotransmitter activated in a voltage-dependent manner and is permeable to Ca²⁺. The voltage dependency is caused by extracellular Mg²⁺, which blocks the ion channel at membrane potentials more negative than -35mV (Cotman et. al., 1989). Because of this voltage-dependency, NMDA currents have a longer time to induction. However, these currents have a longer duration than those of the quisqualate/AMPA receptor. Glutamate has been shown to act predominantly via the non-NMDA receptor while aspartate preferentially activates the NMDA receptor (Gardette and Crepel, 1986). Since 5HT has a greater effect on quisqualate-induced activity than that evoked by NMDA, serotonin may play a greater role in modulating the immediate
firing rate of the neuron. Since the cerebellar nuclei are the source of the cerebellar efferents, modulation of the firing rate of these neurons would cause a modification of the cerebellar output. However, 5HT also effects NMDA-induced activity and thus may effect long term neuronal responsiveness. As stated above, the NMDA receptor is permeable to Ca\(^{2+}\), which has been shown to affect several cellular functions, such as activation of Ca\(^{2+}\)-dependent kinases, that could have long term effects on the responsiveness of the cell. Activation of the non-NMDA receptors (probably the quisqualate/AMPA receptor) depolarizes the membrane, which may induce the removal of the Mg\(^{2+}\) block, and thus allow for the activation of the NMDA receptor (Cotman et. al., 1989). By suppressing quisqualate-induced activity 5HT would decrease the amount of membrane depolarization and thus the critical level of depolarization required for NMDA receptor activation would not be achieved.

Several observations suggest that the physiological effects of 5HT on spontaneous and excitatory amino acid induced activity occurs postsynaptically. First, in the present study the excitatory amino acids were exogenously applied. Thus the suppressive effect of 5HT on excitatory amino acid-activated firing rate suggests an interaction at a postsynaptic site and not a presynaptic decrease in the release of neurotransmitter from cerebellar nuclear afferents. Secondly, several studies were conducted in a low calcium
solution (Gardette et. al., 1987; Gardette and Crepel, 1993), suggesting that the 5HT receptors involved in the observed responses were, at least in part, postsynaptically located.

The present study is the first to determine the interaction between 5HT and synaptic activity. 5HT increases the interval between the shock artifact and the initial spike (initial latency) and the interval between the initial spike and the following spike (interspike interval). By increasing these intervals serotonin is able to decrease the firing rate of the cerebellar nuclear neurons and thus decrease the output of the cerebellum. The increase in the initial latency suggests that 5HT suppresses the neurons responsiveness to the excitatory inferior olivary inputs. The interspike interval represents a period of inactivity induced by the inhibitory inputs from the Purkinje cells. An increase in this interval suggests that 5HT interacts with this inhibitory input. Several possible mechanisms may be involved in 5HT's modulation of the initial latency and interspike interval; First, 5HT may bind to postsynaptic receptors and via intracellular signaling mechanisms, decrease the neuronal excitability. The present study has shown that the 5HT$_{1A}$ receptor mediates at least part of serotonin's effects on cerebellar neuronal activity. The 5HT$_{1A}$ receptor has been shown to activate potassium channels via a G protein-dependent second messenger system (Andrade et. al., 1986; Andrade and Nicoll, 1987). Thus activation of this receptor would
increase potassium conductance, causing the neurons to become hyperpolarized. This would increase the amount of depolarization and time required for activation of the neuron. However, because of the time required for the activation of the ion channels via a second messenger system, this mechanism would be too slow to alter the initial spike latency. Secondly, 5HT may interact with GABA postsynaptically (see below) to decrease the neurons responsiveness, thus inducing the observed increase in the interspike interval. The present study demonstrated that serotonin does interact with GABA and that 5HT potentiates the suppressive effect of GABA on cerebellar nuclear neurons.

The data in the present study demonstrate a complex interaction between 5HT and GABA in which the neuron responds differently depending on whether GABA or 5HT is applied first. One possible mechanism by which 5HT may potentiate the suppressive effect of GABA may involve an interaction between 5HT and GABA at the level of signal transduction in the postsynaptic neuron. Activation of the GABA_A receptor leads to an opening of a Cl⁻ channel, whereas, as stated above, application of serotonin may result in the opening of a K⁺ channels. The net effect is a potentiation of hyperpolarization of the cell (Nicoll, 1988). The GABA_A receptor has been shown to be localized within the cerebellar nuclei (Albin et. al., 1991) and as stated above, the 5HT₁A receptor mediates at least part of 5HT’s effects on cerebellar
nuclear neuronal activity. Thus this mechanism is plausible. A study by Cheun and Yeh (1992) demonstrated that the $\text{GABA}_A$ receptor-activated current could be modulated by norepinephrine in cerebellar Purkinje cells. A similar modulation of GABA activity in the cerebellar nuclei could be induced by serotonin. The observation that a prolonged suppression of neuronal activity occurs only when 5HT is added to a cell pre-conditioned with GABA suggests that 5HT has a greater effect after the cell has been hyperpolarized by the activation of the GABA receptor. However it appears that GABA doesn’t effect the actions mediated by the 5HT receptor in a similar manner, since the present study showed that GABA doesn’t induce a prolonged suppressive effect on neurons pre-conditioned with 5HT. The present study also demonstrated that 5HT and GABA can interact in an additive manner to suppress neuronal activity. One possible mechanism may involve the activation of the $5\text{HT}_{1A}$ and $\text{GABA}_B$ receptors which could induce an interaction at the level of signal transduction on the postsynaptic neuron. It has been demonstrated that $\text{GABA}_B$ and $5\text{HT}_{1A}$ receptors modulate the same potassium channels through a mechanism that involves a pertussis toxin-sensitive G-protein (Andrade et. al., 1986; Nicoll, 1988). Activation of the same potassium channel by both GABA and 5HT would cause a suppression of the cerebellar nuclear neuronal activity in an additive manner. However, at present, there have been no studies that have demonstrated the
presence of the GABA\_3 receptor in the cerebellar nuclei. Since GABA is the inhibitory transmitter utilized by Purkinje cells (the cerebellar cortical neuron that projects to the cerebellar nuclei), the results of the present study suggest that 5HT can modulate the interaction between the cerebellar nuclei and the inhibitory cerebellar cortical inputs.

The effect of 5HT on nuclear neurons circuitry can be summarized in the diagram shown in Fig. 17A. In figure 17A a nuclear neuron is monosynaptically activated by excitatory inputs from brainstem afferents. This initial excitation is followed by a period of suppression which is induced by the inhibitory input from the Purkinje cell. Finally, suppression of the Purkinje cell by cerebellar cortical interneurons causes a disinhibition of the nuclear neuron which is demonstrated as additional excitation. The activity diagramed in 17A was demonstrated in the present studies following the stimulation of the IOC (Fig. 17C). The application of 5HT, to a neuron synaptically activated by the stimulation of IOC, increased the suppressive interval between the initial excitation (Fig. 17D, arrow) and the additional excitatory responses plus decreased the amount of excitation (Fig. 17D). The data from the present study suggest that 5HT induces these observed effects by suppressing neuronal responsiveness to the excitatory inputs (mediated by glutamate and aspartate) and by potentiating the suppressive effect of the inhibitory inputs (mediated by GABA). The present study also suggests that 5HT
mediates these effects via the 5HT1A receptor subtype.

Over the past ten years, it's has become increasingly evident that 5HT utilizes more than one receptor subtype, each with a unique pharmacology and signal transduction mechanism (Fargin et. al., 1988; Maroteaux et. al., 1992; Julius et. al., 1988; McAllister et. al., 1992; Julius et. al., 1990; Schmuck et al., 1994; Maricq et. al., 1991; Matthes et. al., 1992; Kirchgessner et. al., 1992). Thus serotonin can induce different physiological responses depending upon which receptor subtype is present. Darrow et. al. (1990) determined, physiologically, that the 5HT1A receptor mediates some of the effects of serotonin in the young rat cerebellar cortex. In the adult cat, the 5HT1A receptor was shown to mediate 5HT’s effect in the cerebellar cortex (Kerr and Bishop, 1992). Also, in the slice it has been shown that 8-OH-DPAT (a 5HT1A agonist) mimics the inhibitory effect of iontophoretically applied 5HT on cerebellar nuclear neuronal activity (Cumming-Hood, 1990). This response could be blocked by the application of spiperone (a 5HT1A/2 antagonist [Cumming-Hood, 1990]). Thus it was reasonable to determine whether this receptor subtype mediated 5HT’s effect in the cerebellar nuclei. The present study has shown that 5HT does in fact utilize the 5HT1A receptor to mediate at least some of it’s effects in the cerebellar nuclei. At present there is a discrepancy between physiological and anatomical studies as to which 5HT receptor subtype are present in the cerebellar
nuclei. Anatomically the 5HT_{1B} and 2C receptors, but not the 5HT_{1A} receptor, have been localized within the young mouse and rat cerebellar nuclei (Palacios et. al., 1993; Boschert et. al., 1993). In the present study the 5HT_{1B} receptor subtype was not looked at since this receptor subtype has been shown to be present only in the mouse and rat (Heuring et. al., 1986; Hoyer et. al., 1985); it is absent in human, dog, guinea pig, pig, cow, chicken, frog, and turtle. However since the present study focused on the 5HT_{1A} receptor it is possible that 5HT utilizes other subtypes (e.g. 5HT_{2C}) in the cat's cerebellar nuclei.

Even though the serotoninergic afferents that project to the cat's cerebellar nuclei have been shown to arise from brainstem areas which are distinct from those that project to the cerebellar cortex (Kerr and Bishop, 1991; Kitzman and Bishop, 1994), serotonin appears to have similar actions on neurons in these two areas of the cerebellum. One of the brainstem nuclei that give rise to serotoninergic projections to the cerebellar nuclei is the dorsal raphe nucleus. Neurons within the dorsal raphe nucleus (and also raphe magnus and pallidus nuclei) demonstrate a characteristic slow, highly regular spontaneous firing rate (Lanfumey and Jacobs, 1982; Jacobs et. al., 1990; Jacobs and Fornal, 1991). In the cat, the activity of the dorsal raphe neurons has been shown to be closely linked to the sleep-wake-arousal cycle (Jacobs et. al. 1990; 1991). These researchers proposed that the brain
serotoninergic system exerts a modulating influence over its targets so as to coordinate their activity with the animal's sleep-wake-arousal state. Since the dorsal raphe is the major serotoninergic projection to each of the cerebellar nuclei, the rhythmic firing of these neurons would induce a rhythmic suppression of the nuclear neurons. This could help maintain the cerebellar nuclei at a certain activity level depending upon the arousal state of the animal. A more recent hypothesis is that the 5HT system in the brain facilitates motor output and concurrently inhibits sensory information processing (Jacobs and Fornal, 1993). The present study has shown that 5HT has a suppressive, not an excitatory, effect on cerebellar nuclear neuronal activity. This suppressive effect of 5HT on neuronal activity has also been demonstrated in the cerebellar Purkinje cells (Strahlendorf et al., 1984; Kerr and Bishop, 1992). Since the role of the cerebellum is not to initiate motion but rather to co-ordinate activity between various muscle groups participating in a movement, suppression of the activity of these cerebellar nuclear neurons by serotonin, may help to focus the cerebellar output and thus allow for a smooth co-ordinated movement.

Since the origin of serotoninergic afferents to the cat's cerebellar nuclei is distinct from those to the cerebellar cortex these two cerebellar areas do not appear to process all information in parallel. These data also demonstrate that cerebellar function is not rigid but is in fact capable of
being modified in response to changes in the internal environmental. It appears that 5HT is one of the neuroactive compounds which is capable of modifying cerebellar activity and thus motor function.
Figure 7: The Effects of Serotonin on Spontaneous Activity

Oscilloscope traces that illustrate the effect of 5HT on spontaneously active neurons (Figs. 7A–C). Fig. 7A nuclear neuron that demonstrates spontaneous activity. Following the application of 5HT the firing rate decreases (Fig. 7B). The neuron returns to pre-serotonin firing rate following the discontinuation of 5HT application (Fig. 7C). Fig. 7D chart recording demonstrating the suppressive effect of 5HT on unit firing rate.
FIGURE 7

A  Spontaneous

B  Spon + 5HT + 80

C  Spon.

D  5HT +118 +50 +60 +70 +80 +90 +118

1mv

20 msec

50/sec  50 sec
Figure 8. The Effects of 5HT on Activity Induced by Stimulation of the Inferior Olive

Oscilloscope traces illustrating the effect of 5HT on neuronal activity induced by the stimulation of the inferior olive (IO). Fig. 8A demonstrates a spontaneously active neuron. Stimulation of the IOC induces a period of suppression between the shock artifact and additional spikes (Fig. 8B). Concomitant application of 5HT increases the interval between spikes (Fig. 8C). The neuron returns to pre-serotonin firing rate (Fig. 8D). Fig. 8E demonstrates a neuron that displays no spontaneous activity. Stimulation of the IO induces an initial spike, followed by a quite period followed, by a second spike (Fig. 8F). Application of 5HT suppresses all activity after the initial spike and increases the interval between the shock artifact and the initial spike (Fig. 8G). The neuron returns to a pre-serotonin level of activity (Fig. 8H).
FIGURE 8

A. Spontaneous

B. IO + 80

C. IO + 5HT +80 +100

D. IO + 80

E. Spontaneous

F. IO - 50

G. IO + 5HT -50 +30

H. IO - 50

1 mv

20 msec

1 mv

10 msec
Figure 9: The Effects of 5HT on Activity Induced by Inferior Olive Stimulation

Histogram illustrating the effect of 5HT on the initial and interspike intervals induced by stimulation of the inferior olive.
INTERACTION BETWEEN 5HT AND IO STIMULATION

![Graph showing latency (in msec) for different intervals]

- □: IO STIMULATION ONLY
- ■: IO STIM PLUS 5HT

**FIGURE 9**
FIGURE 10: The Effects of 5HT on Aspartate and Glutamate Induced Activity

Oscilloscope traces that illustrate the effects of 5HT on activity induced by the application of aspartate (Figs. 10A-C). Fig. 10A nuclear neuron that demonstrate little spontaneous activity. Following the application of aspartate the neuronal firing rate increased (Fig. 10B). Simultaneous application of 5HT suppresses the augmenting action of aspartate (Fig. 10C). Upon removal of 5HT, neuronal activity returned to pre-serotonin firing rate (Fig. 10D). Figures 10E and F chart recordings that illustrate the effects of 5HT on activity induced by the application of aspartate (Fig. 10E) and glutamate (Fig. 10F).
FIGURE 10
FIGURE 11: The Effects of 5HT on Quisqualate Induced Activity

Oscilloscope traces that demonstrate the effect of 5HT on quisqualate induced activity. Fig. 11A nuclear neuron that demonstrates little spontaneous activity. The application of quisqualate increases the firing rate (Fig. 11B). Fig. 11C 5HT suppresses the quisqualate induced neuronal activity. Upon removal of 5HT, neuronal activity returns to preserotonin firing rate (Fig. 11 D). Fig. 11E chart recording demonstrating the suppressive effect of 5HT on quisqualate induced activity
FIGURE 11

A  Spontaneous
B  Quis -12
C  Quis +5HT
   -12   +40
D  Quis -12

E

5HT  2    5    10    20    30    40    60
Quis  -25

1mv
20msec

50/ sec
50 sec
FIGURE 12: The Effects of 5HT on NMDA Induced Activity

Oscilloscope traces (Figs. 12A-D) and chart recording (Fig. 12F) that illustrate the effect of 5HT on interposed nuclear neuronal firing rate induced by the application of NMDA. Fig. 12A nuclear neuron that demonstrates little spontaneous activity. The application of NMDA augments neuronal firing rate (Fig. 12B). Fig. 12C 5HT suppresses the augmenting effect of NMDA on neuronal firing rate. Upon removal of 5HT, neuronal activity returns to pre-serotonin firing rate (Fig. 12D). Fig. 12E chart recording demonstrating the suppressive effect of 5HT on a NMDA-activated neuron.
FIGURE 12
Figure 13: The Effects of Spontaneous and Excitatory Amino Acid Induced Activity

Summary graph illustrating the effect of 5HT on neuronal activity. In the majority of neurons recorded from, 5HT suppressed spontaneous activity as well as excitatory amino acid induced activity. Two of the neurons demonstrated an increase in firing rate following the application of 5HT, while the remainder of the neurons were not affected by 5HT application.
SPONTANEOUS AND E.A.A. ACTIVATED NEURONS

The Effect of 5HT on Neuronal Activity

FIGURE 13
Figure 14: The Effects of 5HT on Quisqualate Induced Neuronal Activity Versus NMDA Induced Activity

Chart recording demonstrating the effect of 5HT on a interposed nuclear neuron. Fig. 14A chart recording illustrating a single neuron that was activated by both quisqualate and NMDA. 5HT had a greater suppressive effect on quisqualate induced activity when compared to that induced by NMDA. Fig. 14B bar graph illustrating the effect of 5HT on nine different interposed nuclear neurons. Each number illustrates a single neuron that was activated by both quisqualate and NMDA. 5HT had a greater suppressive effect on quisqualate induced activity when compared to that induced by NMDA.
FIGURE 14

A

B

5HT EFFECT ON NEURONAL FIRING RATE

QUISQUALATE INDUCED ACTIVITY
NMDS INDUCED ACTIVITY

PERCENT DECREASE IN FIRING RATE
Figure 15: The Interactions Between 5HT and GABA on Spontaneous and Quisqualate Induced Activity

Chart recordings illustrating the interaction between GABA and 5HT on interposed nuclear firing rate. Fig. 15A GABA suppresses spontaneous activity in a dose-dependent manner. Fig. 15B chart recording illustrating the interaction between 5HT and GABA on a spontaneously active neuron. Figs. 15C,D chart recording illustrating the interaction between 5HT and GABA on two cells activated by quisqualate.
FIGURE 15
Figure 16: The Effects of 5HT₁₄ Agonists on Neuronal Activity

Figure 16A chart recording demonstrating the suppressive effect of 5HT and ipsapirone on nuclear firing rate. Fig. 16B chart recording demonstrating the suppressive effect of 5HT and 8-OH-DPAT on neuronal firing rate.
FIGURE 16
Figure 17: The Physiological Effects of Serotonin on Cerebellar Nuclear Inputs

Figure 17A is a diagram that illustrates the excitatory and inhibitory inputs to the cerebellar nuclei. The bars below the diagram represent the firing rate of the neurons (the vertical bars represent action potentials and the horizontal bars represent the period of suppression induced by the Purkinje cells. Fig. 17B demonstrates a spontaneously active neuron. In figure 17C stimulation of the IOC induces an initial excitatory response (arrow) followed by a period of suppression (closed star), followed by additional excitation. Concomitant application of 5HT increases the interval between the spikes (Fig. 17D).
FIGURE 17

A

Interneuron

PK

DOCN

Brainstem Afferents

B

Spontaneous

C

IOC + 80

D

IOC + 5HT + 80 + 100

1mv

20msec
CHAPTER II REFERENCES


BIBLIOGRAPHY


