RESEARCH ARTICLE

Neuromolecular Imaging and BRODERICK PROBE® nanobiosensors reveal a temporal synchrony in brain rhythms in neural transmission online with movement designs during natural physiology: Temporal asynchrony is imaged online in the same subject during pathology

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ABSTRACT:

Temporal synchrony, discovered in our laboratory using Live Imaging, Neuromolecular Imaging (NMI), and the BRODERICK PROBE® reveals a distinctive rhythmic regularity between neurotransmitter synaptic release and movement frequency in the natural, physiologic state. Using this innovative nanotechnology of NMI and BRODERICK PROBE® nanobiosensors, a point-by-point temporal synchrony was imaged between the synaptic concentration of serotonin (5-HT) released from structures such as dorsal striatum A_9 and ventral striatum A_{10} terminals, basal nucleus, and ventrolateral nucleus accumbens (vlNAcc) in concert with natural open-field behaviors such as ambulating movement and finer movements of grooming, licking, and chewing. Indeed, 5-HT synaptic concentration increased and decreased in parallel and simultaneously with the rise and fall of open-field behavior frequency. Thus, temporal synchrony occurs when the symphony of physiologic neurochemistry and behavior is uninterrupted by drugs, disease, or injury. In contrast, neurotransmitter release and movement frequency are not in concert when monitoring, for example, psychostimulant-induced behaviors produced by cocaine. After cocaine administration, NMI and BRODERICK PROBE® nanobiosensors revealed temporal asynchrony between endogenous 5-HT release at A_{10} terminals, basal stem nucleus, somatodendrites, and VTA and both cocaine-induced ambulations and fine movements in freely moving, male Sprague Dawley animals.

Temporal asynchrony was also seen during intraoperative, *in vivo* studies of neurotransmitter release in anterior temporal lobe epilepsy in a human patient. It is likely that time sensitive asynchrony occurred due to the chemical movement of neurotransmitters away from catecholamine and indoleamine neurotransmitters to excitatory neural transmission via the neuropeptide, dynorphin 1-17, that is readily imaged by NMI. NMI recordings with laminar biocompatible carbon-based BRODERICK PROBE® nanobiosensors were taken at a cortical depth of microns to less than 2 mm for 20-30 minute intervals in the epilepsy patient during resection surgery intraoperatively. Importantly, L-tryptophan (L-TP) was imaged online during the operation showing the lack of the ability of the asynchronous brain to produce 5-HT, the hallmark of the synchronous natural state of physiology. Therefore, L-TP and neuropeptides such as excitatory Dynorphins may serve as neurobiomarkers for pharmaco- and/or gene therapy for neurodegenerative processes. In this manner, temporal patterns may be used to create a dynamic data profile in the clinical setting. Although static neurotransmitter levels are currently the standard, these static parameters become more valuable when empirically studied within the context of movement rather than solely focusing on whether a neurotransmitter *level* has increased or decreased. Not only does this dynamic data provide a more complete portrait of the temporal harmony of physiology, but it may also be used to distinguish the intensity of disease or drug-induced temporal cacophony on specific parts of microneuroanatomy. Therefore, a rhythmic brain, *disrupted*, may well define brain disease.

Keywords: brain, brain circuits, electrical circuits, cocaine, dopamine, epilepsy, movement, seizure, serotonin, neuropeptides, peptides, imaging, biomedical engineering, sensors, nanobiosensors

1. INTRODUCTION:

The 2016 National Survey on Drug Use and Health reported that cocaine usage has remained at a relatively constant rate since 2009 [1], and the 2017 United Nations on Drugs and Crime Report states that cocaine trafficking in both North America and Europe is on the rise [2]. Indeed, cocaine use is prevalent. As with other drugs of abuse, cocaine's reinforcing effects are associated with an increase of dopamine (DA) in the nucleus accumbens [3, 4, 5]; and this DA increase has become the conventional signature for cocaine.

Similarly, in seizures, there is also a disruption in chemical neurotransmitter concentration, leading to abrupt, disproportionately hypersynchronous depolarizations in cortical neurons. In epilepsy, these hypersynchronous depolarizations are recurrent and unprovoked by acute central nervous system or systemic insult [6]. Typically, a lower concentration of the inhibitory neurotransmitter gamma-amino-butyric-acid (GABA) leads to seizure activity. Moreover, in the presence of $GABA_B$ receptor agonists (i.e. Baclofen) that are associated with second messengers, GABA concentrations are decreased presynaptically, leading to hyperpolarization via opening of K^+ channels. [6] Thus, a decrease in presynaptic GABA has become the conventional signature for seizure activity.

Indeed, a static DA increase is associated with cocaine usage, while a static decrease in GABA is associated with epilepsy. Although these signatures for disease states are useful, they do not convey the full pattern of neurotransmitter activity in the disease state. In this paper, we examine a point-by-point temporal synchrony between synaptic neurotransmitter concentration and movement in the natural, untreated and untainted physiologic state. In the physiologic state, synaptic neurotransmitter release re-

flects synchronous movement frequency that is rhythmic with neural transmission processes. In particular, this point-by-point temporal synchrony between natural open-field behaviors such as fine movements and ambulations with 5-HT released from structures including the dorsal striatum A_9 and ventral striatum A_{10} terminals, basal nucleus, and ventrolateral nucleus accumbens (vlNAcc) was demonstrated in freely moving, male, Sprague Dawley rodents using the BRODERICK PROBE®. We focused on the neurotransmitter, 5-HT instead of DA in our animal model as 5-HT release is known to occur in response to cocaine administration and may even play a compensatory or adaptive role in the modulation of cocaine-induced nigrostriatal dopaminergic regulation [7].

Indeed, Jacobs and Azmitia posit that 5-HT's primary function in CNS neuronal circuitry is to facilitate motor output [8]. As discussed by Broderick and Phelix [9], 5-HT somatodendrites exhibit automaticity as they depolarize with regularity by using central pattern generators (CPGs) to produce plateau potentials. The repetitive discharge characteristics of 5-HT neurons occurs in the somatodendritic raphe nuclei before movement onset such as increased muscle tone; and this 5-HT neuronal cell firing is maintained during sustained movements [10] Moreover, raphe nuclei 5-HT cell firing may be phase-locked to repetitive behavioral stereotypic responses, activated preferentially, and is associated with chewing, locomotion, and CPG-stimulated stereotypic behaviors [11].

Thus, in the disease state, we examine temporal asynchrony between 5-HT synaptic neurotransmitter release and movement during cocaine usage, as well as temporal asynchrony between synaptic neurotransmitter release and seizure activity in the epileptic state using NMI and BRODERICK PROBE® nanobiosensors in the anesthetized patient.

We demonstrated temporal asynchrony and/or synchrony here in terms of movement designs such as *chemical* movement from non-toxic neurotransmission to toxic neurotransmission. Hence, for the first time, the neurochemistry underlying normal and pathologic neuronal function in the intact brain of the epilepsy patient during surgery, is reported, *in vivo* at NYU Tisch Hospital under Institutional Review Board Approval with the BRODERICK PROBE® nanobiosensors. The work presented herein then, further advances our original work [12, 13, 14].

2. METHODS:

2.1. Animal Studies:

In adult male Sprague Dawley rodents (n=6), NMI with the BRODERICK PROBE® was used to study the continuous empirical relationship between endogenous 5-HT release in basal ganglia and natural movement behavior, which is directed by basal ganglia *per se*. Specific studies imaging 5-HT release in dopaminergic (DAergic) motor/reward pathways in (1) the nerve terminals, A_9, dorsal striatum (DStr), (2) nerve terminals, A_{10}, ventral striatum (nucleus accumbens) (NAcc) and (3) somatodendrites (cell bodies), Ventral Tegmental Area (VTA), were performed, *in vivo*. NMI enables empirical studies in each animal subject as its own control. Movement was monitored with computerized infrared photobeams, online with imaging neurochemistry, indigenous to each neuroanatomic substrate. Animals were allowed food and water *ad libitum* and were also allowed time to habituate comfortably to their new environment. The new environment was made of plexiglass as was their first home, which was custom made by the Broderick Laboratory and the CCNY Machine Shop; nonetheless, the new environment was a more highly specialized and customized chamber enclosed with copper and manufactured by

San Diego Instruments (San Diego, CA), the Broderick Laboratory (Bronx NY), and the CCNY Machine Shop (Bronx, NY). The Broderick Laboratory installed a commutator circuit to connect with the BRODERICK® detector while the computerized infrared photobeams surrounded the outside of the chamber to assess each movement signal and each movement type at the same time as live neuromolecular signals were videotracking directly from each brain substrate online and instantaneously, essentially within seconds and subseconds. Thus, the copper did not touch or hamper the animals' free movement, exploratory or habituated. Copper was used around the specialized chamber to avoid possible white light frequency; brain signaling was uninterrupted by noise. All electrical systems were engineered grounded for animals' safety and untampered data entry and collection. Thus, NMI signaling was devoid of artifacts. The experimental setup is detailed in Figure 1.

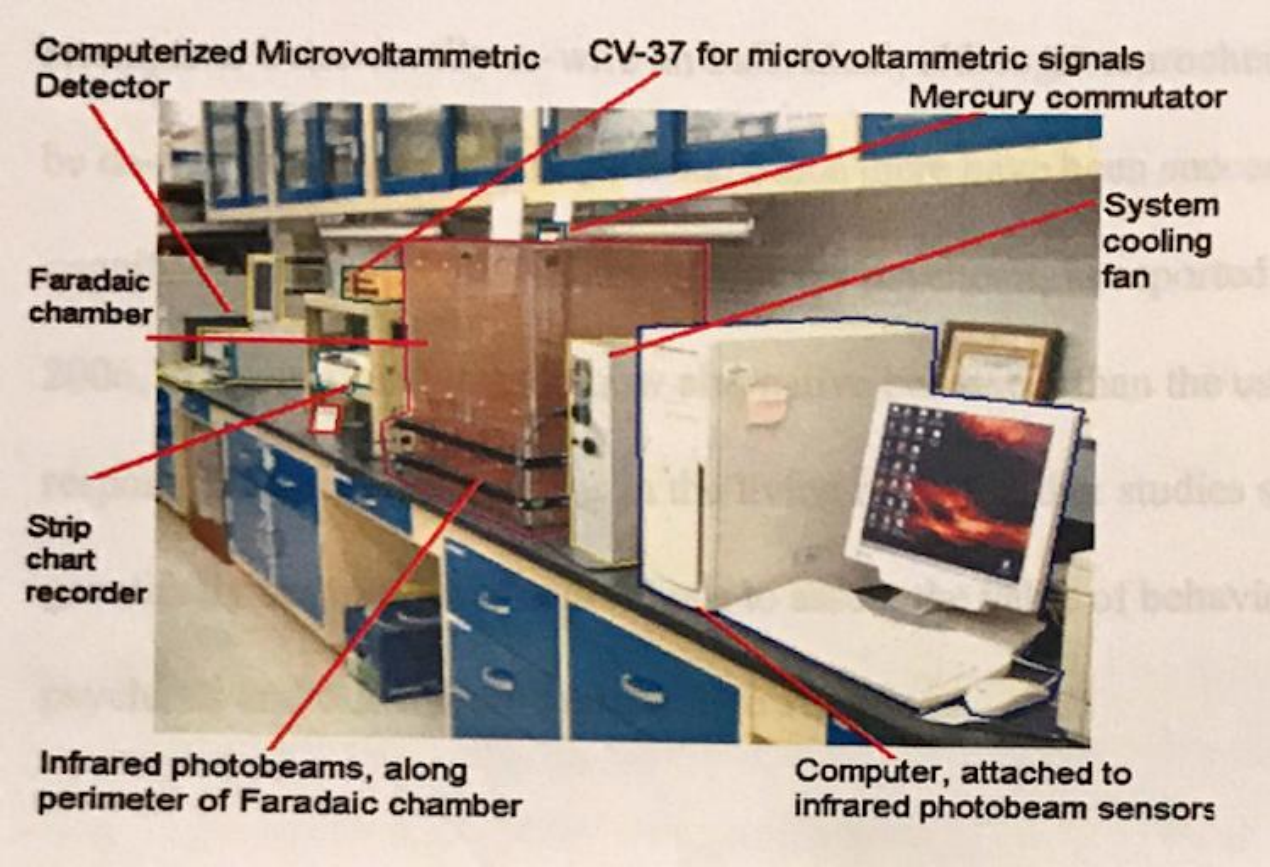


Figure 1: Digital photographs of the NMI analytical devices and behavioral (open-field) equipment used for the simultaneous study of neurochemistry and behavior, not excluding all types of conditioned and unconditioned behaviors.

Therefore, these studies had two control systems. The first control system is the inventive NMI wherein animals are their own control. In the second control system, under one condition, animals did not receive cocaine and were observed for one hour of normal/natural behavior during which nonprescient mammals habituated. Subsequently, after receiving an injection of saline, the animals were studied for neurochemistry

and behavior during a second hour. Then, animals in the experimental group were studied under natural conditions for one hour. Next, these animals received cocaine (10 mg/kg IP or 20 mg/kg SC) and were observed for a subsequent second hour of cocaine-induced, neurochemistry and behavior. In the final study, the animals were observed for a subsequent three hours of cocaine-induced, neurochemistry and behavior. The BRODERICK PROBE® is the *only* technology that images the natural state for comparison with the disease state in the same subject, and this is why intrasubject imaging of temporal synchrony and temporal asynchrony advance the science of medicine significantly.

In these animal studies using carbon-based BRODERICK PROBE® nanobiosensors with the BRODERICK® detector, the sensitivity parameter can be 5 nanoamperes/volt at 10 millivolts/second at a 5 second time constant or a 1 second time constant for the semidifferential circuit electrically connected to the BRODERICK PROBE® (patented and trademarked for both animal and human use [12,13,14,15,16,17]. It is important to note that in the later patents mentioned earlier [15,16], quantum mechanics with a BRODERICK PROBE® protein-based nanobiosensor uses inventive lasers and fiber optical voltaics to image neurotransmitters online in animals and human patients without opening the brain at all, enabling the *patient* to be imaged, freely moving and unrestrained as routinely video-tracked in animal mammalians in the Broderick laboratory and no other. Further intriguing is the patent by Broderick [17], wherein nanoscale science of advanced materials with fullerene nanotechnology uses aspects of semiconductor inventive art to specialize deeper into the exciting exploration of the elusive brain.

2.2. Human Studies:

Using semiderivative and linear voltammetric circuits, NMI, and BRODERICK PROBE® biosensors, the selective detection of specific neurotransmitters and neurochemicals in discrete parts of the intact brain are imaged within a temporal resolution of seconds in a 42-year-old female patient with temporal lobe epilepsy and generalized tonic-clonic seizures. This patient underwent partial cortical resection with intraoperative *in vivo* studies using NMI and BRODERICK PROBE® biosensors. These studies began with EEG monitoring wherein the site of cortical resection was defined with subdural grid epilepsy electrodes that were placed on the surface of the brain with epilepsy strip electrodes placed deeper in the temporal lobe (i.e. hippocampus). Then, biosensors, with a diameter 5 times less than epilepsy depth electrodes for invasive EEG were placed by direct visualization in the exposed cortical region with and without epileptic spike activity in regions destined for resection. About 6 to 10 recordings with γ-irradiated (11.6-12.7 kilograys), laminar biocompatible carbon-based BRODERICK PROBE® laurate biosensors were taken at a cortical depth of microns to less than 2 mm for a 20-30 minute time period. Glial Fibrillary Acidic Protein (GFAP) stains of tissue were post-surgically conducted in order to assess gliosis or other neuronal damage after electrode placement. Based on the pathology report from the NYU surgical team, the BRODERICK PROBE® biosensors did not cause gliosis, sclerosis, brain/neuronal damage, or histiocyte infiltration and are, thus, a safe biomedical tool.

In this human intraoperative study, the BRODERICK PROBE® was used in conjunction with a potentiostat, as well as Micro-Electro-Mechanical Systems (MEMS) technology. Unlike the animal data, the human data was collected using a linear scan instead of a semidifferential circuit due

to the fact that human neurotransmitter concentration is greater and is measured in millamps compared to animal neurotransmitter concentration which is smaller and is measured in nanoamps and picoamps. Thus, the human data produced larger signals at a lower sensitivity than the animal data.

2.3. BRODERICK PROBE® nanobiosensors

Figures 2 and 3 illustrate the BRODERICK PROBE® and all related technologies used to study neural transmission in both the animal and human brain.

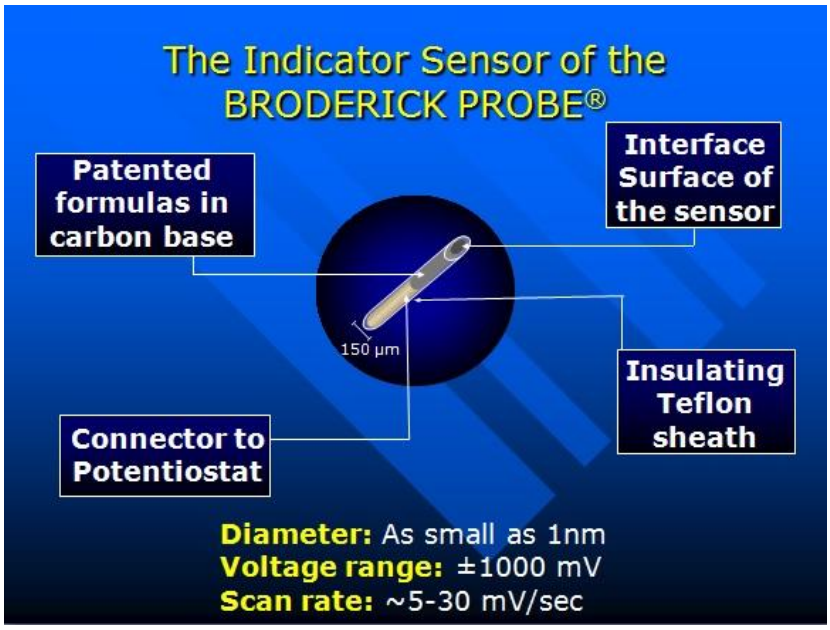


Figure 2: Schematic drawing of just one of several designs for the BRODERICK PROBE® series of nanobiosensors.

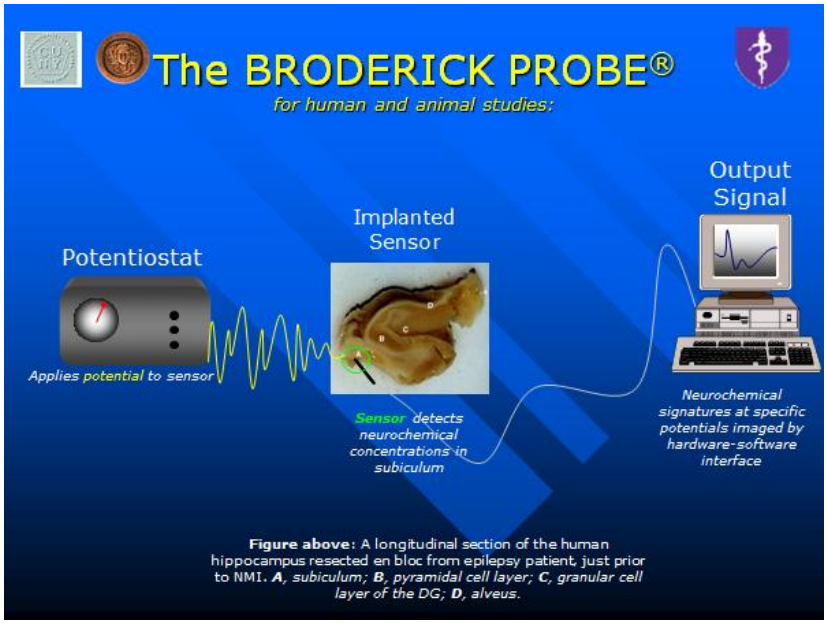


Figure 3: A longitudinal section of the human hippocampus resected en bloc from epilepsy patient, just prior to NMI. *A: subiculum; B: pyramidal cell layer; C: granular cell layer of the dentate gyrus (DG); D: alveus.*

The BRODERICK PROBE® encompasses several formulations of carbon-based biosensors, patented by City University of New York and assigned in part by New York University; all biosensors are assigned to Eazysense Nanotechnologies Incorporated in New York State and were tested in controlled research paradigms [18,19,20, 21,22,23]. A description of specific components for each biosensor formulation, as well as the use, design and applications for these nanobiosensors, are published; detector/potentiostat electrical circuits and associated NMI biotechnology are also published [24,18,25,5,20,26,27,28,29,22]. Broderick patents describe novel inventive constructions and formulations of our nanobiosensor [21,23], which selects an image for a specific neurochemical at an electroactive oxidation/half wave potential using electron transfer kinetics. Subunits of volts and amperes are used to measure the electroactive signature for each neurochemical and is reliant on the electronic circuitry paired with the detector/potentiostat. In these studies, a semidifferential electrical circuit was chosen as several neurotransmitters and neurochemicals can be separately imaged within one minute with each neurochemical imaged within seconds, and recordings can be repeated continuously for hours or longer periods of time. Millivolts are shown on the x-axis and current in nano- and picoamperes is shown on the y-axis using the semidifferential electrical circuit. Specific biosensor properties including the hydrophobicity and hydrophilicity of biosensor formulations within the context of specific interactions with neurotransmitters and neuromolecules was used to derive current from electron transfer kinetics. In the following research, neurotransmitter profiles were imaged using the BRODERICK PROBE® laurate nanobiosensor; and γ-butyrolactone investigations were used to confirm imaging of release mechanisms at the presynapse. Indeed, γ-butyrolactone was used to block neuronal depolarization in specific neuroanatomic sites [30].

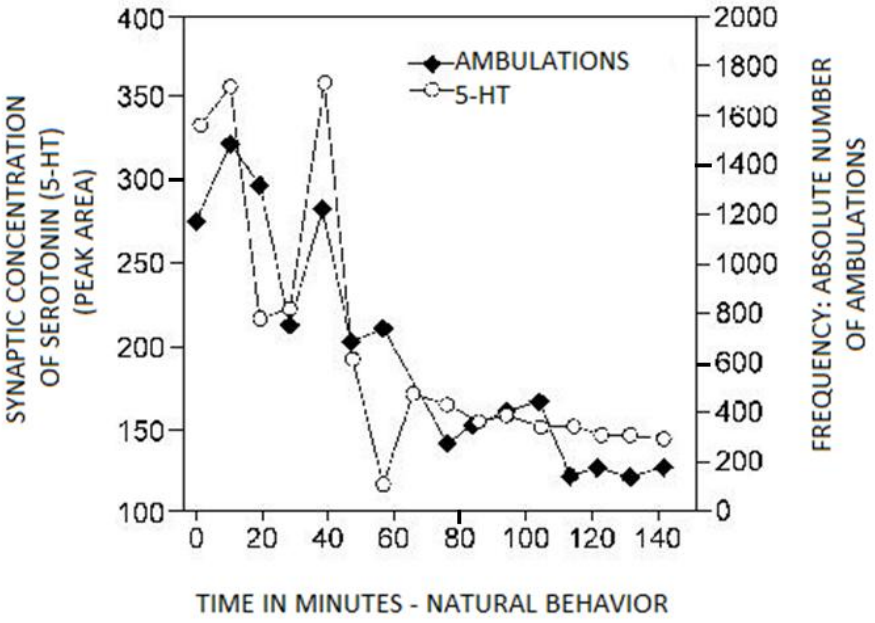
However, this phenomenon was imaged given the caveat that reuptake inhibitory factors at the presynapse can be factored in, despite the fact that reuptake inhibitory mechanisms may not be the main mode of action as often touted.

3. RESULTS AND DISCUSSION:

3.1. Combined Neurochemical-Behavioral Animal Studies:

NMI studies were conducted on freely moving and behaving non-prescient mammals. Concurrently, infrared photocell beam studies of open-field behavior in a novel environment were performed. Thus, each neurochemical data point had a corresponding, correlative behavioral data point. In the control condition, basal 5-HT release was detected at synaptic environments at A_{10} terminal field, vINAcc; at A_9 terminal field, DStr; and at A_{10} somatodendrites, VTA. Figure 4 demonstrates the release of 5-HT at vINAcc (an A_{10} DA terminal field).

A:



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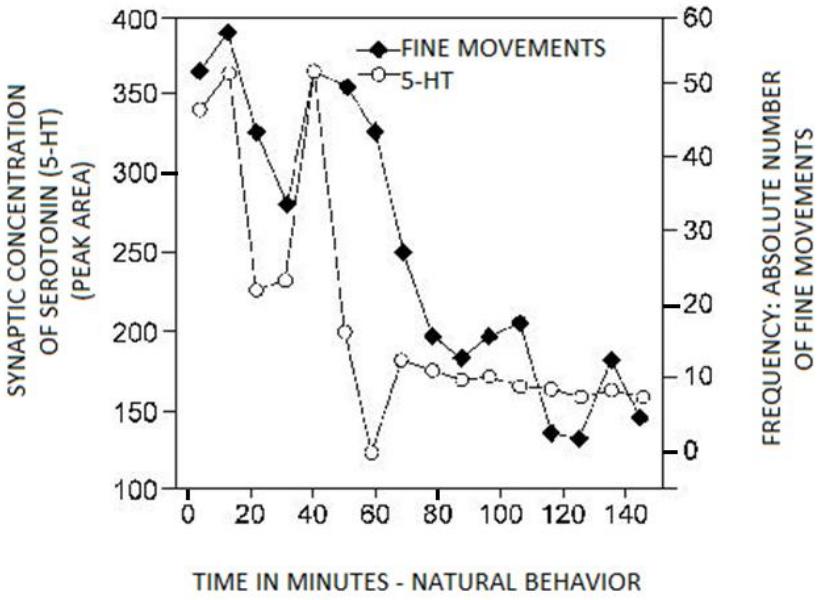
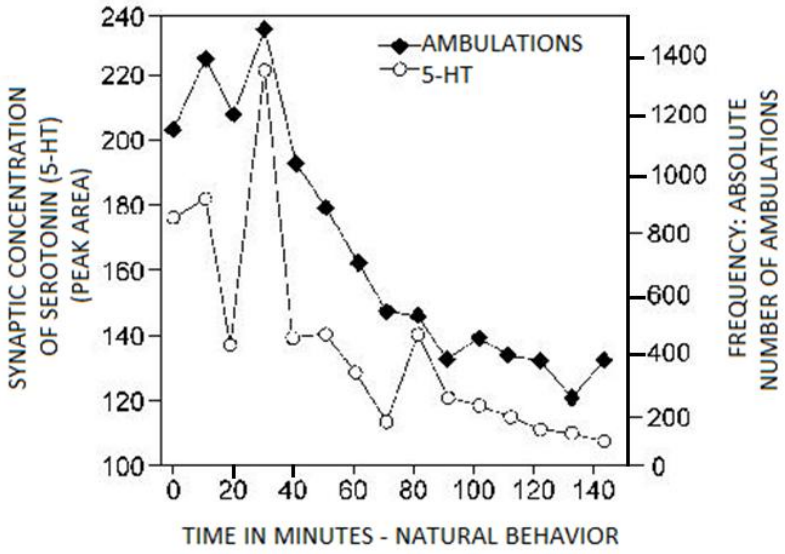


Figure 4. (A) *Ambulations.* Natural neurochemistry and behavior: line graph depicting endogenous 5-HT release (open circles) at basal nucleus, A_{10} terminals, vlNAcc, in real time, while the freely moving, male, Sprague Dawley laboratory rat is actually behaving, during normal/natural movement (first hour) and subsequent post-saline injection habituation behavior (second hour). **(B)** *Fine Movements.* Natural neurochemistry and behavior: Line graph depicting endogenous 5-HT release (open circles) at basal nucleus, A_{10} terminals, vlNAcc, in real time, while the freely moving, male, Sprague Dawley laboratory rat is actually behaving, during normal/natural movement (first hour) and subsequent post-saline injection habituation behavior (second hour).

Figure 5 demonstrates 5-HT release at A_9 terminal field, DStr.

A:



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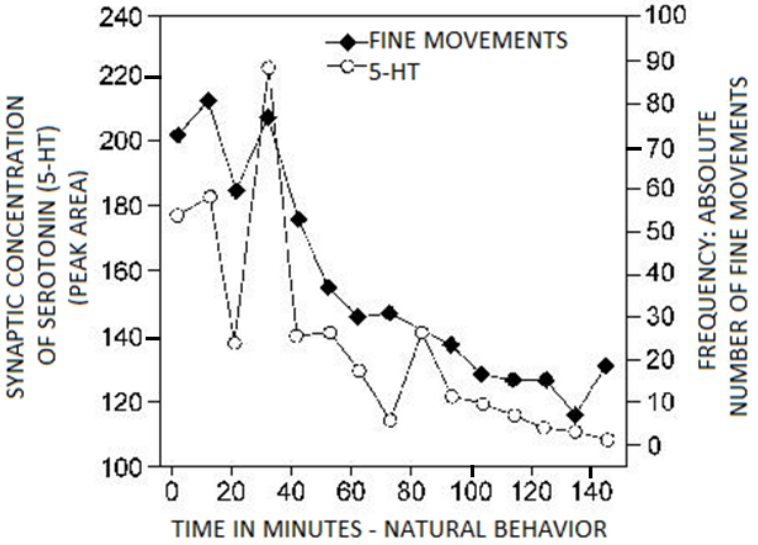


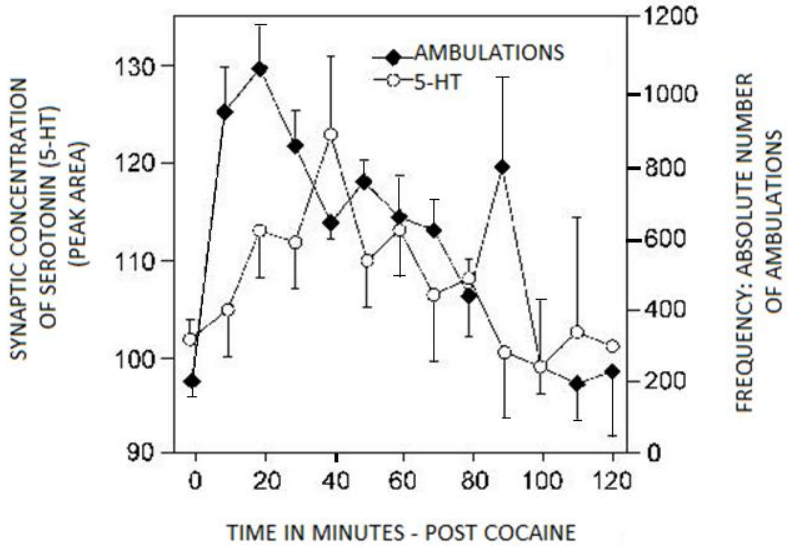
Figure 5. (A) *Ambulations.* Natural neurochemistry and behavior: line graph depicting endogenous 5-HT release (open circles) at A_9 terminals, DStr, detected in real time, while the freely moving, male, Sprague Dawley laboratory rat is actually behaving, during normal/natural movement (first hour) and subsequent post-saline injection habituation behavior (second hour). **(B)** *Fine movements.* Natural (no drug) neurochemistry and behavior: line graph depicting endogenous 5-HT release (open circles) at A_9 terminals, DStr, detected in real time, while the freely moving, male, Sprague Dawley non-prescient mammal is actually behaving, during normal/natural movement (first hour) and subsequent post-saline injection habituation behavior (second hour).

Indeed, the data in Figures 3 and 4 illustrate a direct communication between 5-HT release and open-field behavior. At both the A_{10} terminal field, vINAcc, and the A_9 terminal field, DStr, serotonin release increased, to the same magnitude and at the same time, as each open-field locomotor behavior increased, and there was no demonstration of a temporal gap time lag, even in minutes. Thus, the communication between 5-HT release and naturally occurring behavior was temporally synchronous.

Moreover, at both A_{10} and A_9 terminals, each increase in 5-HT release was simultaneous, and the combined neurochemical-behavioral data were superimposed. Each increase in 5-HT release was concurrent with each increase in movement in the open-field. Therefore, in the control condition, the 5-HT response is positively correlated with each open-field behavioral response.

In the experimental condition, freely moving and behaving non-prescient mammals were injected intraperitoneally with cocaine and 5-HT release at the vINAcc was studied. The data demonstrate that 5-HT increased significantly at the vINAcc. Figure 6 illustrates this cocaine-induced 5-HT release at A_{10} terminal fields versus each cocaine-induced psychostimulant behavior. Thus, it is likely temporal asynchrony in the form of dysfunctional behavior uses distal presynaptic A_{10} circuits and 5-HT-DA interactions.

A:



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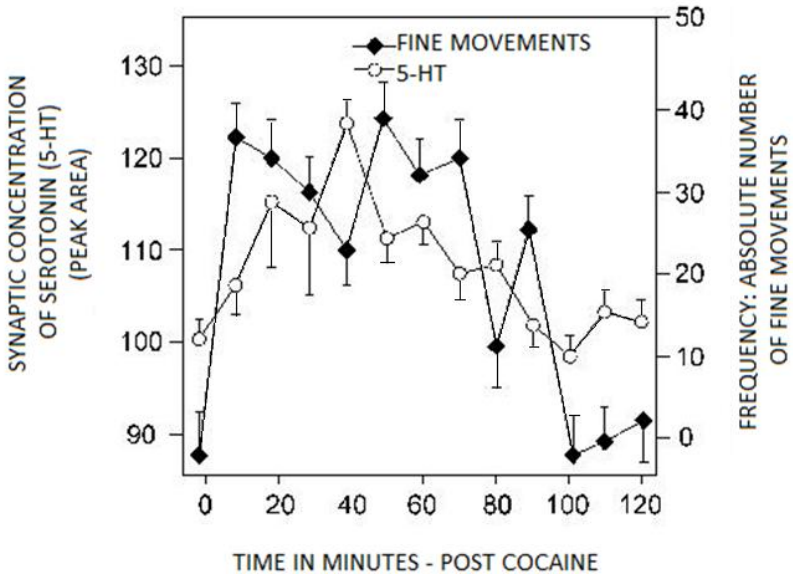
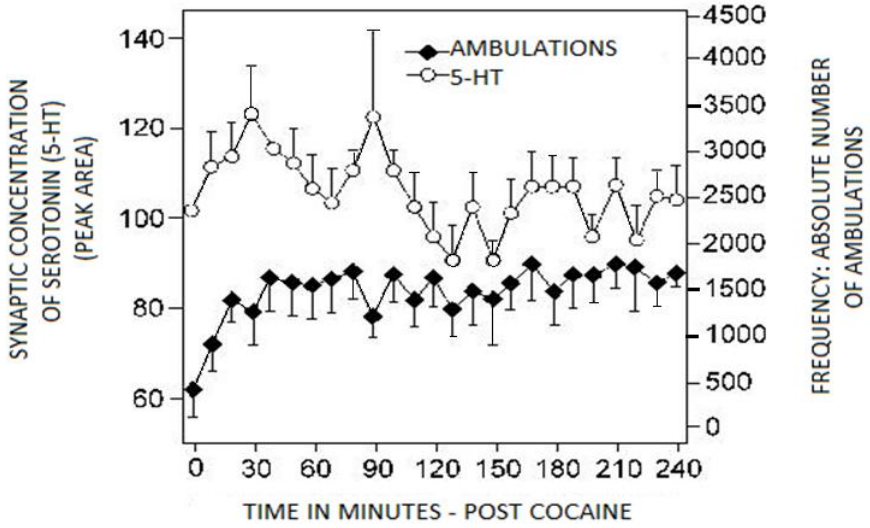


Figure 6. Post 10 mg/kg IP Cocaine: (A) *Ambulations.* Cocaine neurochemistry and behavior: Line graph depicting endogenous 5-HT release (open circles) at basal nucleus, A_{10} terminals,

vlNAcc, in real time, while the freely moving, male, Sprague Dawley laboratory rat is actually behaving, during cocaine-induced behavior (cocaine, 2-h study). Baseline is not shown. **(B)** *Fine movements.* Cocaine neurochemistry and behavior: Line graph depicting endogenous 5-HT release (open circles) at basal nucleus, A_{10} terminals, vlNAcc, in real time, while the freely moving, male, Sprague Dawley laboratory rat is actually behaving, during cocaine-induced behavior (2-h study). Baseline is not shown.

In order to study the 5-HT-DA synaptic interactions in the proximal presynaptic site, we used infrared photocell beam detection of each non-prescient mammal's behavior in the open field to concurrently examine the effects of cocaine on 5-HT release at DA somatodendrites, VTA. Figure 7 demonstrates the effects of cocaine on 5-HT release versus cocaine-induced increases in psychostimulant behavior.

A:



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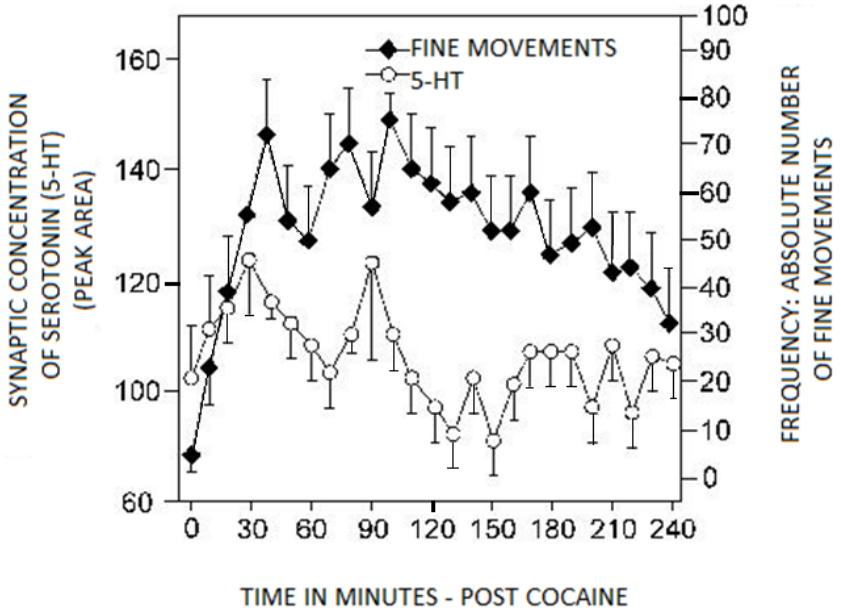

Figure 7. Post 20 mg/kg SC: (A) *Ambulations.* Cocaine neurochemistry and behavior: line graph depicting endogenous 5-HT release (open circles) at basal stem nucleus, DA A_{10}, somatoden-

drites, VTA, in real time while the freely moving, male, Sprague Dawley laboratory rat is actually behaving, during cocaine-induced behavior (cocaine: 4 h). Baseline is not shown. **(B)** *Fine Movements.* Cocaine neurochemistry and behavior: Line graph depicting endogenous 5-HT release (open circles) at basal stem nucleus, DA A_{10}, somatodendrites, VTA, in real time, while the freely moving, male, Sprague Dawley laboratory rat is actually behaving, during cocaine-induced behavior (cocaine: 4-h). Baseline is not shown. Both A and B demonstrate a rhythmic and episodic correlation between movement and 5-HT concentration that is asynchronous.

The data illustrate temporal asynchrony. Each increase in 5-HT release at DA somatodendrites, VTA, is asynchronous with each increase in cocaine-induced locomotion and stereotypy. Resembling the neurochemical profile of cocaine-induced DA release from A_{10} somatodendrites, post-cocaine motor activity exhibits a plateau-like appearance. Indeed, at A_{10} somatodendrites, post-cocaine locomotion and stereotypy are not related temporally to 5-HT release. This phenomenon of temporal asynchrony contrasts the control condition (Figures 3-4) in which natural open-field behaviors of locomotion and stereotypy are temporally synchronous with 5-HT release at A_{10} somatodendrites.

3.2. Human Studies:

Mesial temporal lobe epilepsy occurs in the cortex and has no ascorbic acid or dopamine as biomarkers; instead, there is only norepinephrine as a biomarker. In contrast, neocortical temporal lobe epilepsy occurs in the hippocampus and has both ascorbic acid and dopamine that were successfully separated as biomarkers by the BRODERICK PROBE® (Figure 7). Thus, there are biomarker patterns in epilepsy demonstrating the pathology in asynchrony. Indeed, the BRODERICK PROBE® can distinguish between DA and ascorbic acid (about 40 mV apart), as well as NE and DA (about 50 mV apart) (Figure 7b). Preliminary results showed that catecholamines, a metabolite of dopamine (DA), homovanillic acid (HVA), tryptophan (L-TP), precursor to serotonin (5-HT), dynorphin (DYN), and somatostatin (SRIF) were imaged in neocortex; and the presence of L-TP, peptides and DA metabolites were greater than the presence of monoamines or indoleamines. There is little to no 5-HT present in the image, and what is visualized is likely due to medication given to the patient before surgery. Indeed, the BRODERICK PROBE® and NMI can image the medications given to a patient. (Figures 8B & C)

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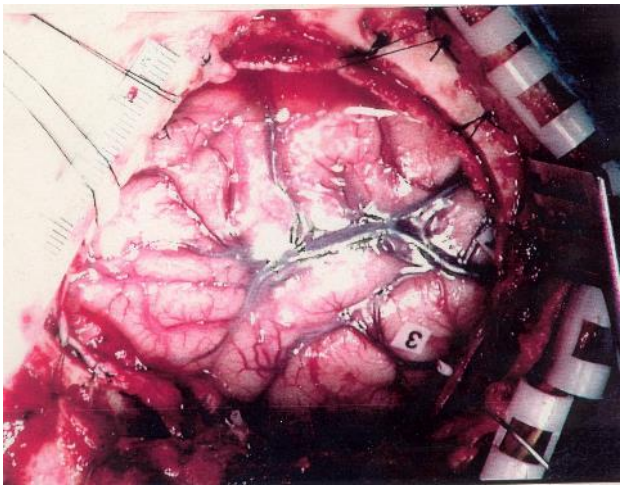

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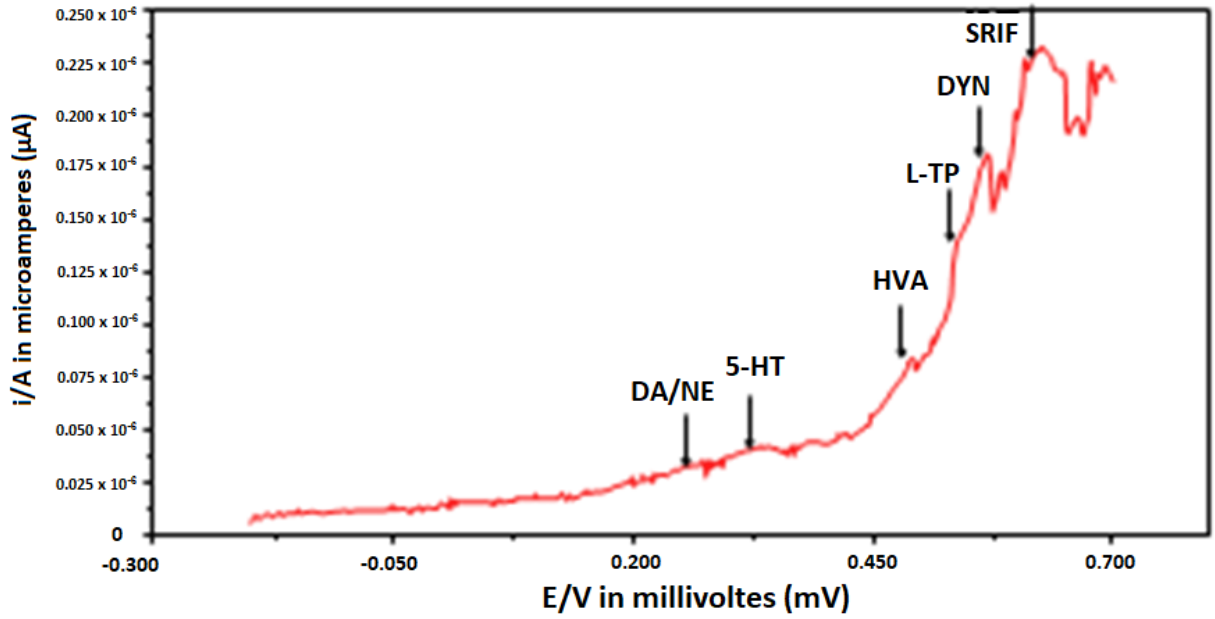

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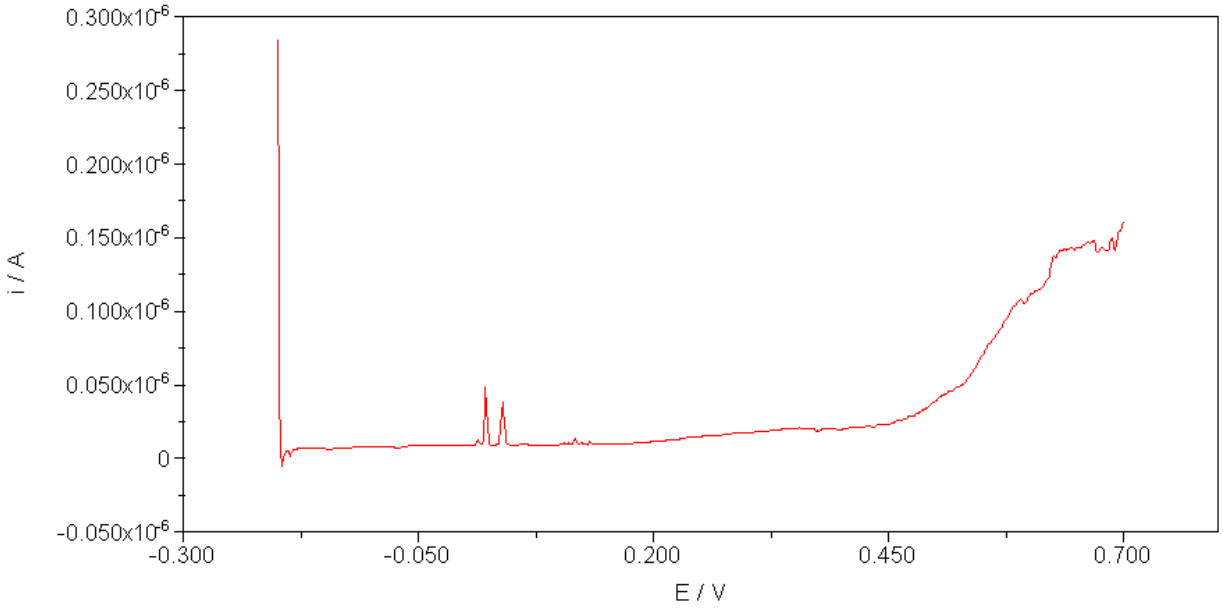

Figure 8: (A & B) Intra-Operative NMI *in vivo* of human epilepsy patient*:* Linear circuit: On the x-axis: oxidation potential in millivolts (mV). On the y-axis: current in µamps. HVA, L-TP, DYN, & SRIF were imaged. L-TP and Peptides were present in higher concentration than the monoamines or indoleamines. There is little to no 5-HT present in the image, and what is visualized is likely due to medication given to the patient before surgery. **(C)** Linear circuit: Human patient studied on 10-23-08. On the x-axis: oxidation potential in mvolts. On the y-axis: current in µamps. HVA, L-TP, DYN, & SRIF were imaged. L-TP and Peptides were present in higher concentrations than monoamines or indoleamines.

4. CONCLUSION:

4.1. Animal Studies:

Endogenous 5-HT synaptic release increased and decreased at dorsal striatum A_9 terminals, dorsal striatum A_{10} terminals, the basal nucleus, and ventrolateral nucleus accumbens (vlNAcc) in parallel with the rise and fall of open-field behavior frequency. Thus, temporal synchrony occurs between natural behaviors and brain neurochemistry in the physiologic state.

Indeed, the data in Figures 3 and 4 illustrate a direct communication between 5-HT release and open-field behavior. At both the A_{10} terminal field, vINAcc, and the A_9 terminal field, DStr, 5-HT release increased, to the same magnitude and at the same time, as each open-field locomotor behavior increased, and there was no demonstration of a temporal gap time lag, even in minutes. Thus, the communication between 5-HT release and naturally occurring behavior was temporally synchronous. Moreover, at both A_{10} and A_9 terminals, each increase in 5-HT release was simultaneous, and the combined neurochemical-behavioral data were superimposed. Each increase in 5-HT release was concurrent with each increase in movement in the open-field. Therefore, in the control condition, the 5-HT response is positively correlated with each open-field behavioral response.

In the experimental condition, freely moving and behaving non-prescient mammals were injected intraperitoneally with cocaine, and 5-HT release at the vINAcc was studied. The data demonstrate that 5-HT increased significantly at the vINAcc. Figure 5 illustrates this cocaine-induced 5-HT release at A_{10} terminal fields versus each cocaine-induced psychostimulant behavior. Thus, it is likely temporal asynchrony in the form of dysfunctional behavior uses distal presynaptic A_{10} circuits and 5-HT-DA interactions.

After cocaine administration at various doses (10 mg/kg versus 20 mg/kg) and with different routes of administration (IP versus SC), there was no positive correlation between endogenous 5-HT release at basal nucleus, A_{10} terminals, and vlNAcc in both cocaine-induced ambulations and fine movements in freely moving animals, which agree with the data presented by other laboratories that observed 5-HT release at the medial NAcc in anesthetized animals [31,32] and awake animals [33,34,35]. Moreover, the data agree with the hypothesis that presynaptic reuptake inhibition and/or release (i.e. increased efflux) of 5-HT occurs despite cocaine-induced decrease in 5-HT somatodendritic cell firing in dorsal raphe (DR) [36, 37]. Therefore, temporal asynchrony occurs between drug-induced behaviors and brain neurochemistry in the non-physiologic state.

Interestingly, the psychostimulant effect of cocaine on dopamine A_{10} somatodendrites in the ventral tegmental area remained rhythmic and episodic but not synchronous. Perhaps psychostimulant effects on temporal synchrony occur with a different pattern of intensity at somatodendrites compared to nerve terminal nuclei.

4.2. Human Studies:

In vivo imaging of the epileptic neocortex during surgery revealed high L-TP and peptides compared to monoamines or indoleamines. Thus, L-TP and peptides may serve as markers for pharmaco- and/or gene therapy for neurodegenerative processes.

Perhaps high L-TP and peptides is indicative of temporal asynchrony induced by the pathologic state of seizure activity. In this manner, temporal patterns may be used to create a dynamic data profile in the clinical setting. Moreover, the dynorphin detected in epilepsy is also evidence of pathology. Somatostatin, on the other hand, may well be a protective neurotransmitter of neural

circuitry in the neurodegenerated brain [38]. In conclusion, L-TP and neuropeptides may serve as neurobiomarkers for pharmaco-and or gene therapy for neurodegenerative processes.

4.3. Dual Species Translational Evidence for Defective Brain Rhythms Factoring in Disease:

Although static neurotransmitter levels are currently the standard, dynamic data from NMI and BRODERICK PROBE® nanobiosensors provide a more complete portrait of the temporal harmony between neurotransmitter concentration and movement in the physiologic state, as well as the intensity of disease or drug-induced temporal cacophony on specific parts of microneuroanatomy in the pathologic state. Data, herein, describe rhythmic temporal synchrony and its converse, arrhythmic temporal asynchrony empirically. Thus, both heuristically and theoretically, these dual species conclusions further demonstrate the critical nature of the rhythmic brain translationally.

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