Neuropathology of Suicide

A Review and an Approach

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ABSTRACT: Neuropathology is one approach to the effort to elucidate the pathophysiology of suicide. Initial neurochemical studies focusing on the roles of serotonin (5-HT) and noradrenaline (NE) abnormalities in brains of suicide victims have been somewhat inconsistent. More recently developed methodologies, including quantitative receptor autoradiography, immunoblotting, immunohistochemistry, cell morphometry, *in situ* hybridization, Northern analysis, solution hybridization/RNase protection assay, reverse transcriptase polymerase chain reaction, and genotyping, which have already been applied successfully in studies of other disorders of brain structure or function, are now increasingly being adopted for postmortem studies of suicide. These new strategies are adding convergent evidence for brain 5-HT and NE dysfunction in the etiology of suicide susceptibility, refining the neuroanatomical localization of this dysfunction, and in addition, implicating heretofore unsuspected candidate neurotransmitter systems in the neuropathological substrates of suicide susceptibility. It is argued here that the confluence of the availability of suitable postmortem samples and this augmentation of our armamentarium of techniques promises the attainment of important new insights into the biological underpinnings of suicide from postmortem research. It is to

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be hoped that this new knowledge might inspire novel pharmacotherapeutic strategies for the prevention of suicide.

INTRODUCTION

Suicide is a serious public health problem, as a significant cause of death for Americans (more than 30,000 per year), striking young adults (adolescents included) and the elderly, in particular,[1] and is a tragic outcome for many patients suffering from mental illnesses and drug addictions (alcohol in particular).[2-4] Ten to fifteen percent of patients with schizophrenia and manic depressive illness die from suicide. No fewer than 50% of completed suicides are alcohol related, whereas the figures for depression approach 80-90 percent. Thus, in addition to the compelling reasons for attempting to elucidate factors contributing to increased susceptibility to suicide, it should become apparent that research on schizophrenia, or for that matter, manic depressive illness, alcoholism, or depression, needs to consider suicide as a state-dependent variable at the time of death. In suicide, as in other disorders where a disorder of brain structure or function has been postulated, a neuropathological approach may prove valuable. The very importance of suicide contributes to the feasibility of research on postmortem brain samples in studying suicide. In the first place, there is, unfortunately, no shortage of victims. A second problem, the fact that suicide is a state rather than a disease, is solvable inasmuch as there are abundant numbers of at least four diagnostic groups (schizophrenics, manic-depressives, unipolar depressives, and alcoholics), examined in medical examiner's offices, who have died from suicide or from other causes.

Although schizophrenia has been dubbed the "graveyard of neuropathology,"[5] the neuropathological approach to studying suicide is affected by many of the same concerns. It is probably safe to say that the failure to elucidate a pathognomonic lesion in either case represents a shortcoming of the research paradigms. An alternative approach, a search for an abnormal neural circuit, might prove more useful in the study of suicide, as has been proposed for schizophrenia.[6] Any hypothesized circuit should probably include the following regions: brain stem serotonergic raphe neurons and noradrenergic locus coeruleus neurons, their projection sites in the prefrontal cortex and the amygdala/hippocampus/mesial temporal lobe, and the hypothalamic-pituitary axis.

The following review will include findings in each of the above four regions as well as recommendations as to useful methods that can be used in their study. Some methodological detail will be devoted to a few of the most recently developed molecular biological techniques that have not yet been applied in this field, in hopes of encouraging researchers to explore their potential utility, especially in light of evidence that genetic factors

may contribute to the pathophysiology of suicide.[7] Of course it should also always be borne in mind that because each of these approaches is limited by particular methodological problems, convergent evidence from multiple approaches yields more compelling support for a given hypothesis. Indeed, this can already be seen in the case of the consistent implication of 5-HT and NE systems in suicide by a combination of findings from neurochemical, cell morphometric, receptor binding, immunohistochemical, and *in situ* hybridization techniques.

GENERAL CONSIDERATIONS IN POSTMORTEM BRAIN STUDIES

We have reviewed extensively elsewhere,[8] in the context of methodological issues in postmortem studies of mental illness, a variety of concerns that need to be addressed in carefully designed studies, which will be briefly summarized here. The optimal source of postmortem brains for the study of suicide is from medical examiners' offices, where the vast majority of cases are autopsied. Several major advantages of this source include the relatively short postmortem intervals and the availability of normal controls from the same facility in order to control for a host of postmortem artifacts, as well as a preponderance of younger subjects relative to other sources. Ideally, controls should be matched to suicides for age, gender, race, postmortem interval, and storage time. Psychiatric diagnosis is another critical factor in postmortem studies. Medical records, family interviews, and police reports can provide useful information in this regard. History of alcohol or substance abuse is an important factor for which to control.

It is important in these cases to obtain toxicological screens of urine, blood, or brain. A new research approach, segmental hair analysis, offers the promise of providing a longer, objective history of substance abuse. It is also now possible to screen brain samples for the presence of HIV-1. In our own brain collection at the Clinical Brain Disorders Branch of the National Institute of Mental Health Neuroscience Center, we are currently screening for HIV sequences by polymerase chain reaction (PCR), using primers and probes from highly conserved regions of the HIV-1 genome (Perkin Elmer Corp., Norwalk, CT), followed by Southern blot or dot/slot blot analysis. Finally, samples from brains to be used in studies also need to be examined histologically for evidence of neuropathology, such as cerebrovascular disease, tumors, and Alzheimer's disease or other dementias. We use Bielschowsky's silver stain on 5 μm-thick paraffin-embedded sections to evaluate the number of mature neuritic plaques and neurofibrillary tangles,[9,10] compared to age-matched controls, to exclude cases of Alzheimer's disease.

GROSS STRUCTURAL MORPHOLOGICAL STUDIES

Somewhat ironically, an early postmortem finding of gross structural abnormality in brains from suicide victims was the serendipitous result of the use of a suicide control group in a schizophrenia research study. Altshuler *et al.*[11] found a unilateral (right sided) reduction in the area of the parahippocampal cortex in suicide victims and a bilateral reduction in schizophrenia patients. This finding aided, in turn, the interpretation of an earlier postmortem schizophrenia study,[12] which has been oft miscited as evidence for neuropathology localized in the left hemisphere in schizophrenia victims. In this latter study,[12] the schizophrenic victims had a thinner parahippocampal gyrus on the left side, but not on the right side, relative to depressed patients. Actually, victims of schizophrenia had similar left and right hemisphere measures, whereas depressed patients had a thinner right parahippocampal gyrus relative to their left hemisphere, and relative to the right hemisphere in normal victims. These results could not be correctly interpreted without knowing the normal anatomy. Parahippocampal gyrus width was not asymmetric in normal victims, and was roughly equivalent to the size of the left hemisphere of depressed patients. The correct interpretation was, instead, that schizophrenia involved bilateral reduction in parahippocampal thickness, whereas the parahippocampal pathology in affective disorders was confined to the right hemisphere.

NEUROTRANSMITTERS AND RELATED ENZYMES AND METABOLITES

Neurochemically oriented postmortem studies of neural substrates involved in affective disorders have been driven primarily by neuropharmacological knowledge. Depletion of monoamines by reserpine mimics depression[13,14] and, conversely, what are believed to be the common mechanisms between effective pharmacotherapeutic treatments for depression are monoamine oxidase inhibition, blockade of monoamine reuptake, and more recently, selective 5-HT reuptake inhibition. Indeed, early measurements of 5-HT levels in homogenates from the brain stems of suicide victims did appear to confirm a 5-HT deficit.[15–17] However, there were also several failures to replicate these findings.[18–21] Similarly, attempts to assess brain stem 5-HT function by measurement of the metabolite 5-hydroxyindoleacetic acid (5-HIAA) yielded reports of either deficits[18,19] or no significant change[16,17,21] in suicide victims. In the terminal fields of 5-HT neurons, as well, reports have included reduced 5-HT in the putamen[22] and hypothalamus,[21] increased 5-HT in the globus pallidus and putamen,[21] or no change in 5-HT in the frontal cortex,[20–25] hippocampus, amygdala, and temporal cortex.[21,22,25] Measurements of 5-HIAA have detected a

reduction in the nucleus accumbens,[21] an increase in the hippocampus[26] and amygdala,[22] or no significant change in the frontal/prefrontal cortex,[19,21-23,25,27,28] basal ganglia, hippocampus, amygdala, and temporal cortex[21,25] in suicide victims. Activity of the enzyme MAO A, for which 5-HT is a preferred substrate, has also been found not to differ in the prefrontal cortex (Brodmann areas [BA] 8 and 9) from suicide victims.[29]

Attempts to measure brain stem NE function by measuring postmortem NE levels from suicide victims have been uniformly negative.[16,18,30,31] Increases have been found in the temporal cortex (BA 38),[32] hippocampus, and hypothalamus;[30] no change was found in the ventral septum, the bed nucleus of the stria terminalis, the nucleus accumbens, and the mammillary bodies,[33] and a reduction has been detected in the putamen.[19] One attempt to assess prefrontal cortical (BA 10) NE function in suicide by measuring the metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) was negative.[28] Yet, as will be seen below, convergent evidence from a variety of other more recently developed methods does implicate NE, as well as 5-HT dysfunction in the neuropathology of suicide. It may well be that these discrepant results are due to the difficulties of matching for such variables as age, manner of death, and postmortem interval, and by the postmortem instability of these neurotransmitters and their metabolites.

Two other neurochemical findings from postmortem suicide studies, that of reduced hypothalamic glutamine, but normal GABA and glutamate levels in caudate, nucleus accumbens, frontal cortex, amygdala, and hypothalamus[34] and of reduced prefrontal cortical (BA 10) and caudate neuropeptide Y,[35] also need to be further examined with additional approaches. Recently, an attempt to assess the function of the phosphoinositide signal transduction system by measuring G-protein levels, finding elevated $G\alpha s$ levels in BA 10, and decreased $G\alpha i2$ levels in BA 8/9 in postmortem brains from suicide victims, has further implicated neurochemical abnormalities in the prefrontal cortex (BA 8, 9 and10) in suicide.[36]

RECEPTOR BINDING

Alterations in neurotransmitter and neuromodulator receptor density may play an important role in the neurobiological basis of affective disorders, in general, and suicide, in particular. Such changes may either be primary to the illness or secondary compensation for distant pathological changes within the central nervous system. In addition, the mechanism of action of many antidepressant medications, like most psychotropic drugs, is through binding to pre- and/or postsynaptic receptors and/or transporters. Many experimental techniques are now available for the study of pre- and postsynaptic receptor systems. One of the oldest techniques is radioligand binding to either tissue homogenates or slide-mounted tissue sections.

Tissue homogenate and slide-mounted tissue section studies have different strengths and weaknesses. Tissue homogenate preparations involve the use of blocks of tissue, from which cellular membranes are isolated and studied. By the nature of the technical processing, neurons, astrocytes, oligodendroglia, endothelial cells, and any other cell type present in the tissue block will contribute to the membrane isolate. This limits the anatomical localization of receptors at a cellular level. In addition, many structures have a heterogeneous distribution of receptors. For example, in the human striatum, the ventral putamen has double the density of β_1-adrenergic receptors compared to the dorsal caudate,[37] a difference that would be missed in whole striatal homogenate preparations. However, membrane homogenate studies allow the pharmacokinetics of the receptor to be studied in detail, including the total receptor density per milligram of wet tissue and the relative affinity of a compound for a particular receptor through Scatchard analyses. In addition, the linkage of receptors to second messenger systems can be explored.

Research based on tissue sections is more useful from an anatomical perspective. First, this allows precise anatomical localization within the rostrocaudal extent of a given structure, although membrane isolates from micropunches of tissue can sometimes achieve fairly good localization. Second, slide-based research allows localization of receptor binding to subregions within a structure, such as patch versus matrix in the striatum or the cell clusters within layer II of the entorhinal cortex. Third, silver grain analysis permits a fine degree of cellular localization of receptors, differentiating those that are expressed on glial elements from those on neurons. The anatomical benefits of tissue sections must be weighed against the difficulties in performing pharmacokinetic analyses on such a preparation. Pharmacokinetic studies, as discussed in the previous paragraph, are much more easily performed and probably more accurate when tissue homogenates are used. The characterization of a receptor and/or compounds that bind to a receptor is much better examined on tissue homogenates. The anatomical localization of receptors is much better studied on tissue sections.

Postmortem brain studies of suicide have relied upon both tissue homogenate and slide-mounted tissue section techniques. Through the application of these techniques, a number of findings have been reported. The primary focus of radioligand-binding experiments has been 5-HT and NE receptor systems, although cholinergic and corticotropin-releasing factor receptors also have been studied.

Within the 5-HT system, 5-HT_1, 5-HT_2, and 5-HT presynaptic uptake sites have been extensively examined. Experiments are sometimes difficult to compare due to the use of different brain regions, different dissection techniques within similar brain regions, and the use of different compounds to assay a particular receptor. For example, tritiated spiperone has been used in many studies of the 5-HT_2 receptor system, but some

important studies have relied upon iodinated LSD or tritiated ketanserin. The relative affinity for a particular receptor varies between compounds. Additionally, few compounds bind to only one receptor, especially at concentrations needed to measure B_{max}. Different compounds may have markedly different affinities for secondary binding sites. With these limitations in mind, several interesting results have come forward.

Probably the most consistent finding in postmortem studies of suicide has been an increase in the density of 5-HT_2 receptors in the prefrontal cortex, in studies encompassing BA 8, 9 and 10.[38–44] It is noteworthy that in at least one of these reports,[41] the finding may have reflected a state-dependent effect, because 5-HT_2 receptor density was higher in schizophrenic patients dying of suicide relative to those dying of natural causes; nonpsychotic suicide victims did not differ from normal controls. Other studies reported no differences in these regions between normal controls and suicide victims[26–28,45] for 5-HT_2 receptors, or, more specifically, for 5-HT_{2A} receptor binding.[46] One group even reported decreased 5-HT_2 receptor density.[47] Hrdina *et al.*[40] found a similar increase in the amygdala. Cheetham *et al.*[45] did not find an increase in the amygdala but did detect decreased 5-HT_2 binding in the hippocampus in antidepressant-free suicides, although this was not found in the total suicide group,[45,48] nor was it found for 5-HT_{2A} receptors.[46] Joyce *et al.*,[49] assaying the hippocampus, the temporal cortex posterior to the temporal pole, and, separately, the entorhinal cortex, detected no change in 5-HT_2 receptor density in suicides. Overall, although the reason for some discrepant findings is not clear, there does appear to be a significant increase in prefrontal 5-HT_2 receptor density in suicide.

A decrease in the 5-HT uptake site has been found in the prefrontal cortex (BA 11, 45, and 46;[50] BA 10;[41] BA 8 and 9[44,51]) of suicide victims. However, several other groups reported no change ("frontal cortex";[26,28,52] BA 9;[40] BA 9, 10, 11 and 32;[53] BA 8 and 9;[54] BA 9 and 10;[55] and BA 10[56]) or even an increase[57] in binding to the 5-HT uptake site in the prefrontal cortex of suicide victims. It is perhaps noteworthy that the latter negative study[56] was based on antidepressant-free depressed suicide victims, raising the possibility that some of the reported elevations could be secondary to antidepressant treatment history. Decreases in the density of the 5-HT uptake site have also been seen in temporal and entorhinal cortices,[49] the postcentral gyrus, the insular cortex and claustrum[53] and the hypothalamus,[58] although the latter was not confirmed by other studies.[40,52,53] An increase has been reported in the hippocampus[53] but there have been failures to replicate this.[23,55] No differences were seen in the amygdala,[40,53] cingulate,[52,53] temporal (inferior, medial, superior temporal and temporal gyri and parahippocampal gyrus),[53] or occipital cortices (BA 17 and 18[41] or in the lateral occipitotemporal gyrus[53]), midbrain (substantia nigra,[53,55] red nucleus,[53] ventral tegmental area, perirubral area[55]), caudate, putamen, globus pallidus, thalamus, or substantia innominata.[53]

Taken together with the data on 5-HT_2 receptors, it appears overall that there may be an upregulation of postsynaptic 5-HT receptors in the frontal cortex and a downregulation of the presynaptic uptake sites in suicide. This suggests a dysregulation of 5-HT neurotransmission, with a decrease in bioavailability. The receptor changes may be a secondary compensation for diminished 5-HT release in the prefrontal cortex. However, a caution that must be borne in mind in interpreting these studies is that in using [^{3}H]paroxetine as a ligand we may not be selectively assaying 5-HT uptake sites. Mann *et al.*[25] used cold sertraline to define the specificity of binding of ^{3}[H]paroxetine and inferred from their results that it was not the high-affinity 5-HT uptake that was reduced in the prefrontal (BA 9) and temporal (BA 38) cortices from suicide victims, but rather a high-affinity nontransporter binding site without a known functional role.

5-HT_1 receptor changes are not as clearly substantiated in suicide. Arango *et al.*[50] reported increased 5-HT_{1A} receptor density in the prefrontal cortex in suicide victims (especially in BA 45). In the temporal lobe, Joyce *et al.*[49] also found increased density of 5-HT_{1A} receptors, in temporal and entorhinal cortices. However, other studies of 5-HT_1 binding in the prefrontal cortex (frontal cortex,[26,28] BA 8 and 9[27] or BA 10[59]), temporal cortex (BA 38)[59] or hippocampus,[26] or of 5-HT_{1A} receptors in the prefrontal cortex (superior and middle frontal gyri,[60] BA 8 and 9,[61] BA 10[46,59]), temporal pole (BA 38),[59] or hippocampus[46,60] found no changes. One study[59] actually detected lower 5-HT_1 receptor binding in the amygdala (reduced affinity) and hippocampus (reduced density). The role of 5-HT_{1A} receptor changes in suicide needs additional clarification. Binding to 5-HT_{1C} receptors has also been measured and found to be elevated in the hippocampus in suicide victims.[48]

In addition to studies of the 5-HT system, the adrenergic system has also come under scrutiny in radioligand-binding studies, but the results are quite confusing. Most of the research has focused on α_2-adrenergic receptors. Meana *et al.*[62,63] found increased α_2-adrenergic receptor density in prefrontal cortex (BA 9). Andorn[64] reported an absent low-affinity component of α_2-adrenergic receptor binding in the "prefrontal" cortex in suicide victims, whereas Arango *et al.*[32] detected no change in the prefrontal cortex (BA 9) or temporal pole (BA 38). In the hypothalamus, as well as in the cerebellar cortex of suicide victims, Meana *et al.*[63] once again found an increased density of α_2-adrenergic receptors. Ordway *et al.*[31] noted increased agonist, but not antagonist, binding to α_2-adrenergic receptors in the locus coeruleus from suicide victims. Additional studies are needed to resolve these conflicting reports.

α_1-Adrenergic and β-adrenergic receptors, and the NE uptake site have come under limited scrutiny. Arango *et al.*[32] found an increased density of α_1-adrenergic receptors in the prefrontal cortex (BA 9). However, another study[65] reported decreased α_1-adrenergic binding in the medial

and inferior prefrontal cortex; the orbital, superior frontal, and postcentral gyri; the superior, medial, and inferior temporal gyri and caudate nucleus; though not in thalamus, hippocampus, parahippocampal gyrus or amygdala from suicide victims. Mann *et al.*[42] first reported increased β-adrenergic receptors in the prefrontal cortex (BA 8 and 9). Arango *et al.*[38] also detected increased β-adrenergic binding in the prefrontal cortex (BA 9) in suicide vicitms. They also found an increase by receptor autoradiography but not for binding in homogenates in the temporal pole (BA 38). The prefrontal finding replicated, in part, a report of Biegon and Israeli,[66] who also found increased binding in the superior frontal and cingulate cortices. By contrast, De Paermentier *et al.*[67] noted a decrease in β- and β_1-adrenergic receptor density in the temporal pole (BA 38), and in β_1-adrenergic receptor density in more posterior temporal regions (BA 21/22), whereas other groups found no change in binding to β-adrenergic receptors in the prefrontal cortex (frontal cortex[28,57] or BA 8 and 9[68]) of suicide victims. One study reported a decrease in the anterior superior frontal gyrus of the frontal pole.[69] Paul *et al.*[58] found no differences in the density of the NE transporter in the hypothalamus of suicide victims. More study of these receptors is needed to place them in the proper biological context in suicide.

A few miscellaneous receptor systems have been the subject of radiopharmacology studies in suicide. Although Meyerson *et al.*[57] reported elevated muscarinic cholinergic receptor binding in the frontal cortex from suicide victims, Kaufmann *et al.*[70] did a survey of muscarinic receptor binding in the prefrontal (BA 10) cortex, hypothalamus, and pons and found no changes. Nemeroff *et al.*[71] reported decreased corticotropin-releasing factor binding in the frontal cortex (BA 10 and 11) in suicide victims. Nowak *et al.*[72] found alterations in the NMDA receptor complex (a reduced proportion of high-affinity, glycine displaceable binding to the glutamate recognition site, while neither the potency nor the maximum efficacy of glycine in inhibiting this binding was affected) but not in binding to the NMDA receptor-coupled ionophore in the prefrontal cortex (BA 10) from suicide victims. Gross-Isseroff *et al.*[73] reported increased mu opioid receptor density in cingulate, precentral, postcentral, and superior temporal cortices from young (but not old) suicide victims and an overall increase in young and old suicide victims in the prefrontal inferior frontal gyrus. Independent replication of these studies is needed before they can be given strong weight in the pathophysiology of suicide.

Clearly, many important clues have been uncovered by receptor binding approaches, regarding the abnormalities in the central nervous system of individuals who commit suicide, particularly in the 5-HT system in the prefrontal cortex. The mechanism of action of one of the most effective classes of antidepressants, 5-HT uptake inhibitors, is consistent with the postmortem findings. Additional parts of the neural axis need to be explored, especially the brain stem source of the ascending monoamine

cell groups. Furthermore, other neurotransmitter receptor systems deserve additional scrutiny.

IMMUNOLOGICAL METHODS

As already noted, several lines of evidence have implicated the 5-HT and NE neurotransmitter systems in the pathophysiology of suicide. Thus, proteins involved in the biosynthesis, regulation, and turnover of 5-HT and NE are of particular interest for researchers in this field. Two approaches are most commonly employed to visualize and quantify proteins: the first, immunoblotting techniques, or Western blots, are used to measure levels of proteins and determine their molecular mass. The second, immunohistochemical methods, are commonly used to visualize proteins in tissue sections, characterize their regional distribution, and localize them to specific neural structures. Both methods have been used successfully in postmortem brain specimens from subjects with disorders such as schizophrenia and bipolar illness. It is surprising, therefore, that these methods have thus far been so infrequently used in the study of postmortem brain specimens from suicide victims.

Two studies from the same group[74,75] examined imidazoline receptor proteins, in both the platelets of live patients and in the prefrontal cortex of suicide victims, using Western blots. Imidazoline receptor proteins have been identified in the brain and appear to interact with the NE system. For example, these receptors are thought to inhibit NE release in the brain stem and have been shown to stimulate tyrosine hydroxylase (TH) production in chromaffin cells. The authors examined postmortem brain specimens from 13 suicide victims and 11 controls and reported an increase in a 45 K_d imidazoline receptor protein and a reduction in a 29/30 K_d receptor protein in the prefrontal cortex of suicide victims compared to normal controls. More studies examining imidazoline receptors and their interactions with the catecholaminergic systems in the human brains are needed, however, in order to clarify the putative role of these receptors in the pathophysiology of suicide. One other recent study of suicide using Western immunoblotting found increased detergent-extracted levels of specific isoforms of neuronal cell adhesion molecules and L1 in hippocampi from suicide victims,[76] but the relevance of these proteins to monoamine or other neurotransmitter function remains to be elucidated.

A study by Biegon & Fieldust[77] used immunohistochemical methods and antibodies directed against TH, the rate-limiting enzyme in catecholamine biosynthesis, and against dopamine β-hydroxylase (DBH), the last enzyme in NE biosynthesis, to examine the locus coeruleus of six suicide victims and six matched controls. Using semiquantitative methods, these authors reported a significant reduction in the optical density of TH immunoreactivity in the locus coeruleus of suicide victims compared to

controls. By contrast, the number of TH-immunoreactive neurons was unchanged, suggesting a reduction in the level of TH per neuron. No differences were found in DBH immunoreactivity between the two diagnostic groups. This study provided the first evidence for the involvement of TH in the locus coeruleus of suicide victims. Unfortunately, the small number of cases examined and the preliminary nature of the quantitative data limit the value of these results. Furthermore, contradictory results were subsequently reported. In the caudal locus coeruleus, Ordway *et al.*[78] found elevated TH in suicide victims in nine age-matched pairs by quantitative Western immunoblot. Obviously, further studies are required to address this apparent discrepancy.

Thus, despite long-standing interest in the role of the NE and 5-HT neurotransmitter systems in suicide, only a handful of investigators have used immunoblotting and immunohistochemical techniques to ask questions about the role of various receptor proteins, enzymes, and transporters critical to these systems in the postmortem brains of suicide victims. Some of the technical obstacles that may have hindered these studies include the development of specific and sensitive antibodies and the degree of preservation of levels of proteins detectable by these antibodies during the postmortem interval. These obstacles are not insurmountable, however, as demonstrated by postmortem studies of schizophrenic subjects.

CELL MORPHOMETRY

An elegant recent study by Arango *et al.*[79] has tested the hypothesis gleaned from the earlier neurochemical studies, that brain stem NE innervation to the forebrain is deficient in suicide cases, by using computerized quantitative morphometric techniques to quantify the number and density of pigmented NE cell bodies in the locus coeruleus in postmortem specimens. They found a reduction by 23% in number and by 38% in density of these neurons in suicide victims, without any overall reduction in the length or volume of the locus coeruleus . One especially valuable aspect of this method is that the reduction could be anatomically localized to the rostral portion of locus coeruleus, which provides innervation to the cortex rather than to the hindbrain.

MOLECULAR BIOLOGICAL APPROACHES

DNA supplies information needed to code for brain proteins by directing the transcription of messenger RNA (mRNA), which, in turn, directs the synthesis of a protein. From a reductionist perspective differential gene expression is determined by the molecular interaction

between genes (biological predisposition) and the environment (experience). Measurement of mRNA in postmortem brain studies has been shown to be feasible. RNA has been shown to be stable over a wide range of postmortem intervals,[80] thus providing a reliable signal in most human brain samples. Many cDNA templates for probe synthesis are readily available for human mRNAs, allowing for application of standard molecular biological techniques and uniform tissue processing. Moreover, there are practical reasons for measuring mRNA to quantitatively assess a protein's level of function: RNA is located in the cell body, which allows verification of the cellular source of synthesis; and while absolute levels or activity of a protein are influenced by both synthesis and degradation, levels of mRNA may provide a more sensitive index of fluctuations in regulation of that protein in cell function.

In situ hybridization histochemistry uses the hybridization of a radiolabeled probe, which is complementary to a part of the mRNA sequence. By virtue of a radioactive standard curve, the level of mRNA to which a probe hybridizes can be semi-quantitatively measured.[81] Inasmuch as this method is applied to sections of tissue, in contrast to those below that are based on homogenates, the relative advantages and disadvantages are somewhat analogous to those described for receptor binding methods above, in terms of the trade-off between anatomical resolution and sensitivity. As is true for quantitative receptor autoradiography, *in situ* hybridization measurements can be made both at the regional (in film autoradiography) and cellular (in emulsion-dipped slides) levels of analysis. Especially considering that this strategy has already proven valuable in postmortem schizophrenia studies (see ref. 82 for review), it is disappointing that it has thus far not been much applied in suicide research. López *et al.*[83] found elevated pro-opiomelanocortin mRNA density per cell but no difference in glucocorticoid receptor mRNA in the anterior pituitary from suicide victims. A study of mRNA for the 5-HT transporter in dorsal and median raphe nuclei failed to find any abnormality in depressed suicides.[55] Decreases have been found in mRNAs for the 5-HT_{1A} receptor and the glucocorticoid receptor in the hippocampus from unmedicated suicide victims with a history of major depression.[84] Ironically, unexpected findings have come from schizophrenia studies, which employed suicide victims as a control group to measure neurochemical systems not previously implicated in suicide. An elevation was found in cholecystokinin (CCK) mRNA in the dorsolateral prefrontal cortex (BA 9) in suicide victims, but not schizophrenics.[85] Reductions were found in both suicide victims and schizophrenia patients, in the hippocampus, for *l*-proline transporter mRNA, used as an index of glutamate function.[86] The ratio of patch to matrix prodynorphin mRNA in the caudate was elevated in the caudate nucleus in suicide victims relative to both schizophrenia patients and normal controls (who did not differ), whereas mRNAs for proenkephalin, and D1 and D2 dopamine receptors were not abnormal.[87]

There are now available additional, somewhat different, strategies for measuring mRNA in postmortem homogenates, which have not, to date, been exploited in studies of suicide but which are described briefly here in the interest of proposing them as valuable candidates for utilization in future studies. As is true of all methodologies, each has strengths and drawbacks, and, ultimately, convergent evidence from multiple approaches to a particular question may provide more compelling support for any given hypothesis.

RNA can be studied after purification from other cellular constituents. Specific RNAs can be measured by Northern analysis,[88] which can also provide a useful confirmation of the specificity of any probe used to study RNA by other methods, such as *in situ* hybridization histochemistry or RNase protection assay. Inasmuch as a Northern analysis requires that RNA be separated by size on a gel before hybridization, the size of the transcript RNA can also be determined, allowing for detection of any variation in transcript length due to alternative splicing. The mRNA length may influence the size of the protein which is coded for by that RNA, and ultimately determine the functional capabilities of that protein. Although Northern blots do not allow for quantitation of the absolute amount of mRNA, relative differences in RNA signal across samples have been obtained by optical density readings from hybridization signals detected on films. Northern analysis sensitivity is limited by the amount of RNA that is resolvable on the agarose gel; thus it is not as sensitive as the solution hybridization/RNase protection assay.[89] Because the absolute amount of RNA is determined by an RNase protection assay, results across experimental runs can be combined, and molar abundance of different transcripts can be compared. Because only a small piece of RNA is measured, a sample with a slight amount of degradation can usually produce a clean signal in the RNase protection assay, whereas it may not in Northern analyses.

All PCR protocols require DNA as the starting material. However, to investigate changes in RNA, a reverse transcriptase (RT) enzyme can first be used to generate DNA from the sample, which can then be amplified and measured by interpolation along a standard curve with varying known amounts of starting DNA. RT-PCR can be performed either on homogenates or *in situ* on slide-based sections.[90] Procedures for RT-PCR have improved, yet they are limited by high variability and the inability to quantitate absolute amounts of RNA because of the exponential nature of the reaction. It is, however, the most sensitive of the above techniques and is very powerful in detecting the presence of a transcript in brain tissue.

Finally, some recent association studies have suggested that genotyping from postmortem brain tissue may also prove a valuable tool in suicide research. In a study of alcoholic violent offenders from Finland, there appeared to be an association between genotype for tryptophan hydroxylase, the rate-limiting enzyme in 5-HT synthesis, and suicide attempts.[91] Curiously, it was the relatively rare allele for a polymorphism at the intron

7 of the gene that distinguished those offenders who did not commit suicide from those who did, whereas attempters did not differ from normal controls, suggesting that this allele protects against suicide in some fashion in otherwise violent individuals. Another study of a polymorphic Ava II restriction site for the tryptophan hydroxylase gene in suicide attempters, compared to normal controls, failed to find an association for this polymorphism.[92] A further critical role for genotyping studies in suicide research should be that of elucidating subsets within the population of suicide victims. For just one example, the recent association of anxiety-related traits with a polymorphism in the 5-HT transporter gene regulatory region[93] should prove extremely interesting to study among suicide victims.

CONCLUSIONS AND FUTURE RESEARCH PROJECTS

It is evident, we hope, from the above that the field of postmortem suicide research is in the (perhaps enviable!) position of having at hand a wealth of both samples and new methods to clarify and extend the often contradictory existing literature. At the risk of repetition, one take-home message from the above should be the value of convergent evidence from multiple approaches to this problem. In future studies, we hope more careful control of such factors as the contribution of psychiatric diagnosis or the history of medication will help to shed light in the areas where results to date seem discrepant. Postmortem interval also appears to be a critical factor to carefully control for and could account for some of the apparently contradictory receptor-binding results in the literature.[94] Finally, the potential clinical utility of such basic knowledge should be reinforced. As noted above, several serendipitous findings in this field could ultimately inspire new pharmacotherapeutic strategies that might treat the underlying predisposition and hence, ideally, help patients avoid suicide. Even in the case of neurotransmitters, such as glutamate, now implicated in suicide along with the monoamines, which is ubiquitous in brain function, more detailed knowledge of neuroanatomically specific abnormalities and of the normal tonic levels of turnover of these neurotransmitters in various brain regions could lead to novel treatment approaches. Drugs with increasingly specific affinities for receptor subtypes, or other mechanisms of action (effects on enzymes, release, or reuptake), are now being developed. Such drugs could have relatively preferential central nervous system effects in the desired direction and location (*e.g.*, see Gale & Casu[95]) for a given neurotransmitter, for which an imbalance in the brain plays a role in producing increased susceptibility to suicide. Lastly, molecular biological approaches have been relatively underutilized, thus far, in postmortem suicide researach. They offer enor-

mous promise for elucidating the pathophysiology of suicide, with the hope that this progress will lead to new treatments and prevention.

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