Dysfunction of Astrocyte Connexins 30 and 43 in Dorsal Lateral Prefrontal Cortex of Suicide Completers

Carl Ernst, Corina Nagy, Sangyheon Kim, Jennie P. Yang, Xiaoming Deng, Ian C. Hellstrom, Kwang Ho Choi, Howard Gershenfeld, Michael J. Meaney, and Gustavo Turecki

Background: Suicide is an important public health problem that results from the interaction of both psychosocial and biological factors. Although it is known that particular neurobiological processes underlie suicidal ideation and behavior, there still remains limited knowledge about the specific factors involved.

Methods: To explore the neurobiology of suicide we generated microarray data from dorsal lateral prefrontal cortex (DLPFC) in each of 28 male French-Canadian subjects (20 suicide completers). These results were followed up in a larger French-Canadian sample ($n = 47$, 38 suicide completers) and in microarray data available from the Stanley Foundation ($n = 100$, 36 suicide completers). To investigate the molecular mechanisms of this finding, we performed RNA interference and electrophoretic mobility shift assays. Animal behavioral experiments were done to control for drug and alcohol effects.

Results: We found reduced expression of Cx30 and Cx43 in DLPFC of suicide completers. We identified a previously unknown function for Sox9 as a transcription factor affecting expression of Cx30 in brain.

Conclusions: These results suggest that alterations of astrocyte connexins might be involved in the suicide process and provide further evidence implicating astrocytes in psychopathology.

Key Words: Astrocyte, connexin, genetics, prefrontal cortex, Sox9, suicide

Suicide is the leading cause of death in young adults, affecting people regardless of race, gender, or socioeconomic status. In a 2000 report the World Health Organization estimated that over 1 million deaths occurred by suicide in that year, a statistic overshadowed by the rate of suicide attempts, estimated to occur at a rate 25× that of suicide completion (1,2). Studies aiming to understand the biology of suicide have proceeded since the mid-1960s, when it became clear that suicide was not just a reactive response to a life event (3) but also associated with underlying biological processes (4). To date, different brain systems and their corresponding genes have been associated with suicide (reviewed in Ernst *et al.* [5]), yet independent and consistent replication of most findings remains elusive.

Traditionally, astrocytes have been considered as the support cells of the central nervous system, although more recently studies indicate that astrocytes play a number of different functional roles. For example, astrocytes express functional neurotransmitter receptors, respond to synaptic activity, and form part of the tri-partite synapse where they can modify communication between neurons (6). A growing number of studies have suggested that astrocytes might also be implicated in psychiatric disorders. In schizophrenia, astrocyte numbers are reported to be reduced (7,8) independently of the possible effects of antipsychotic medication, whereas other studies suggest glial cell dysfunction without a reduction in actual cell counts (9–11). In major depressive disorder, studies have suggested astrocytic cell count alterations in a number of different brain regions (12–15). Astrocytic dysfunction might also be directly involved in major depression (MD). For example, glia-derived factors stimulate synaptogenesis (16–18), where altered synaptic connections could underlie the pathology of depression. In addition, recent important evidence indicates that riluzole, a drug thought to upregulate the (SLC1A3/A2) glial glutamate receptors (18), is effective in the treatment of MD (19).

In the current study, we used samples from Quebec and an independent replication sample to study the neurobiology of suicide. As a starting point, we took advantage of microarray data from dorsal lateral prefrontal cortex (DLPFC) from suicide completers and control subjects to assess brain gene expression changes. Through a series of complementary studies, here we present evidence of reduced expression of astrocyte connexins Cx30 and Cx43 in DLPFC of suicide completers. We further identified a transcription factor of previously unknown function in astrocytes, Sox9, as a potential regulator of Cx30.

Methods and Materials

French-Canadian Subjects

Brain tissue for this study was obtained from the Quebec Suicide Brain Bank (QSBB) (http://www.douglasrecherche.qc.ca/suicide). A case was considered a suicide when classified as such by the office of the coroner. Control subjects were individuals who died suddenly and, identically to suicides, could not have undergone any resuscitation procedures or other type of medical intervention. All subjects were male persons of French-Canadian origin (identified by determining whether both sets of grandparents were born in Quebec and spoke French) (Table 1).

Brains that are part of the QSBB are collected in partnership with the Quebec Coroner's Office and undergo a psychological autopsy to retrieve phenotypic information. Briefly, brains are collected after consent is obtained from next-of-kin, and samples from brain tissue, peripheral blood, and urine are collected for toxicologic analysis. Immediately after a death, families are contacted, and the person

From the McGill Group for Suicide Studies (CE, CN, JPY, XD, GT), Department of Psychiatry (CE, ICH, MJM, GT), Douglas Hospital Research Institute, and the Sackler Program for Epigenetics and Psychobiology at McGill University (MJM, GT), McGill University, Montreal, Quebec, Canada; Department of Psychiatry (SK, KHC, HG), University of Texas Southwestern Medical School, Dallas, Texas; and the Stanley Laboratory of Brain Research, Rockville, Maryland.

Address correspondence to Gustavo Turecki, M.D., Ph.D., McGill University, Douglas Hospital Research Centre, Pavilion Frank B Common, Rm. F-3125, 6875 LaSalle Blvd., Verdun, Montreal, QC Canada H4H 1R3. E-mail: gustavo.turecki@mcgill.ca.

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Table 1. Descriptive Variables of French-Canadian Subjects

	Microarray Sample		Validation Sample	
	Suicide $n = 20$	Non-Suicide $n = 8$	Suicide $n = 38$	Non-Suicide $n = 9$
Age, yrs	36.8 ± 14.1	42.1 ± 13.7	45.4 ± 14.3	49.1 ± 22.3
Gender (M/F)	20/0	8/0	38/0	9/0
PMI (h)	27.3 ± 7.2	23.1 ± 4.9	54.7 ± 19.4	58.6 ± 24.2
Brain pH	6.57 ± .24	6.51 ± .19	6.70 ± .26	6.53 ± .27
Toxicology[a]	1/3/4/0	0/0/0	6/3/3/2	0/0/0/0
Death	13/1/3/3/0/0[b]	8[c]	27/8/0/0/1/2[b]	9[c]
DSM-IV[d]	3/1/12/2	0/0/0/0	8/3/8/1	0/0/0/0

The continuous variables represent the mean ± SD; no significant differences were found between any variables.
DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, 4th edition; F, female; M, male; PMI, postmortem interval.
[a]Antidepressant present/cocaine present/ethanol present /diazepam present.
[b]Alcohol dependence/cocaine dependence/major depression/bipolar I.
[c]Hanging/intoxication/gunshot/asphyxiation/drowning/jumping.
[d]Accidental death.

best acquainted with the deceased is recruited to undergo a series of structured interviews. The interviews are supplemented with information from archival material obtained from hospitals, the Coroner's Office, and social services. After the interviews, clinical vignettes are produced and assessed by a panel of clinicians to generate DSM-IV diagnostic criteria. All procedures in this study were approved by the ethics review board of our institution.

Stanley Foundation Subjects

The Stanley external replication sample consisted of 36 suicides and 64 non-suicides (Table 2). There were 44 subjects with bipolar disorder, 45 subjects with schizophrenia, and 11 subjects with MD. There were 22 suicides in the bipolar disorder sample, 10 suicides in the schizophrenia sample, and 4 in the MD sample. Brain tissue was dissected from DLPFC, as described here (http://www.stanleyresearch.org/brain/).

Neuroanatomy

Brains were analyzed, processed, and dissected into different regions on the basis of histological maps (20,21) and gyri/sulci landmarks at the QSBB. We define the DLPFC as encompassing Brodmann areas 9, 10, 11, 44a, 45, 46, and 47, and only gray matter was used from the left hemisphere. Three different cortical regions (from the lateral occipital gyrus, postcentral gyrus, and middle temporal gyrus) were also sectioned and Nissl-stained to detect any signs of pathology. After dissection, brain sections were flash frozen in isopentene and stored at −80°C. Brain pH measurements were performed as described (22).

Table 2. Descriptive Variables of the External Validation Sample of Subjects From the Stanley Foundation

	Suicide $n = 36$	Non-Suicide $n = 64$	p
Age, yrs	40.4 ± 10.3	45.6 ± 9.9	.01
Gender (M/F)	19/17	43/21	—
PMI	35.3 ± 18.4	32.5 ± 15.5	ns
Brain pH	6.4 ± .3	6.4 ± .3	ns
Alcohol[a]	6/9/5/3/4/9	15/15/6/7/7/13	—
Drug abuse[b]	12/5/5/4/5/5	33/4/4/5/6/10	—
Antipsychotic[c]	25/11	51/13	—

The continuous variables represent the mean ± SD.
F, female; M, male; ns, not significant; PMI, postmortem interval.
[a]Alcohol; little or none/social/moderate drinking in past/moderate drinking in present/heavy drinking in past/heavy drinking in present.
[b]Drug abuse; little or none/social/moderate drug use in past/moderate drug use in present/heavy drug use in past/heavy drug use in present/unknown.
[c]Antipsychotic treatment; Yes/No.

Microarray Analysis

Microarray analysis was performed with the Affymetrix Human Genome (HG)-U133 Plus 2.0 chip in DLPFC and processed with robust multi-array averages (RMA). No RNA extracted from human brain was used with an RNA integrity number value < 6 (23). In Stanley foundation samples, RMA-normalized microarray data from four independent studies performed in DLPFC were downloaded from the Stanley Medical Research Institute database (http://www.stanleyresearch.org/brain/). Microarray data from the same platform, Affymetrix Human Genome U133 Set A (HG-U133A), were used to avoid platform-to-platform variation. Two to three microarray chip datasets were generated from each Stanley patient sample ($n = 100$ total subjects). Duplicate microarray datasets were treated as technical replicates. Expression data were analyzed with Genesis 2.0 (GeneLogic, Gaithersburg, Maryland) and AVADIS (Strand Genomics, Redwood City, California). Several RNA integrity measures were used in the screen to detect samples with poor RNA quality before final analysis. Microarray quality control parameters included: noise (RawQ), consistent scale factors, and consistent β-actin and glyceraldehyde-3-phosphate dehydrogenase 5′/3′ signal ratios (> .3 and > .5 for all probes, respectively). Microarray data were filtered by fold change (> 2) and p value (< .01). False Discovery Rate analysis was performed with BRB-array tools (http://linus.nci.nih.gov/BRB-ArrayTools.html) on the default setting ($p < .1$).

Animal behavioral experiments, western blot details, and RNA analyses methods can be found in Supplement 1.

Sox9 Knockdown Experiments

Short hairpin RNA (shRNA) plasmids directed at rat Sox9 were assayed in a rat astrocyte cell line (CCL-107; ATCC) maintained in F-12K medium containing 2.5% fetal bovine serum, 15% horse serum, and 1% penicillin-streptomycin. We used Sure_silencing shRNA (Invitrogen, Carlsbad, California) directly from the manufacturer to transiently knockdown Sox9. The clone with the most efficient knockdown capabilities of Sox9 had the cloneID: GAGCGACAACTTTACCAGTTT. All transfections were performed with Lipo-