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Toll-Like Receptors in the Depressed and Suicide Brain

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Abstract

Abnormalities of the immune function in depression and suicide are based in part on the observation of increased levels of proinflammatory cytokines in the serum and postmortem brain of depressed and suicidal patients. We have examined if abnormalities of the innate immune receptors, known as Toll-like receptors (TLRs), in the brain are associated with depression and suicide, since the activation of these receptors results in production of cytokines. Of all the TLRs shown to be present in humans, TLR3 and TLR4 appear to be unique and important in brain function. We have determined the protein (by ELISA method) and mRNA expression (using qPCR) of TLR3 and TLR4 in the postmortem brain (dorsolateral prefrontal cortex [DLPFC]) of 22 depressed suicide victims, 11 non-depressed suicide victims, 12 depressed non-suicide subjects and 20 normal control subjects. We found that the mRNA expression of TLR3 and TLR4 was significantly increased in DLPFC of depressed suicide victims and in depressed non-suicide subjects, compared with controls. However, the protein expression of TLR3 and TLR4 was significantly increased in depressed suicide victims, but not in depressed non-suicide subjects compared with controls. The observed abnormalities of proinflammatory cytokines in the brain of suicide victims may be related to an abnormality of TLR3 and TLR4 over-expression. To our knowledge, this is the first study of TLRs in the brain of psychiatric subjects.

Keywords

Toll-like receptors (TLRs); cytokines; depression; suicide; postmortem brain; innate immunity

1. Introduction

There are several lines of evidence that suggest abnormalities of the immune system in depression and suicide. This is based on the observation that proinflammatory cytokines, which are released from immune cells as a result of inflammation or stress, are abnormal in the serum of patients with depression [see reviews by Dowlati et al. (2010)]. Other evidence for the involvement of cytokines in depression is derived from studies suggesting that the

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administration of interferons (IFN), or other cytokines, induces depression in patients with chronic hepatitis C, multiple sclerosis, or some forms of cancer, as reviewed by Capuron et al. (2003).

There is some direct and indirect evidence suggesting a relationship between immune dysregulation and suicide. Steiner et al. (2008) have found increased microgliosis in the postmortem brain of suicide subjects compared with normal control subjects. Lindqvist et al. (2009) found increased levels of interleukin (IL)-6 in cerebrospinal fluid (CSF) of suicide attempters compared with normal control subjects, and Janelidze et al. (2011) observed elevated levels of IL-6 and tumor necrosis factor (TNF)- α and decreased levels of IL-2 in suicide attempters. That cytokine levels may also be abnormal in the brain of suicide victims is substantiated by a report of Pandey et al. (2012) who observed increased protein and mRNA expression levels of IL-1 β , IL-6, and TNF- α in the prefrontal cortex of teenage suicide victims compared with normal controls. Shelton et al. (2010), using a microarray method, found that a number of pro- and anti-inflammatory cytokines are upregulated in postmortem brain of subjects with major depressive disorder (MDD). Taken together, these studies suggest abnormalities of the immune function in depression and in suicide in general, and an abnormality of cytokine levels in the postmortem brain of suicide victims in particular.

Although the reasons or mechanism of increased proinflammatory cytokines in the brain of suicide victims as reported by these investigators are not clear, this increase in cytokines may be related to abnormalities of the innate immune receptors, known as Toll-like receptors (TLRs), in the brain (Hanke & Kielian, 2011). There is now increasing evidence to suggest that the central nervous system (CNS) organizes innate immune response during systemic infection and neuronal injury (Crack & Bray, 2007).

Because of the important role TLRs play in neuronal function, and because of their involvement in the production of cytokines and chemokines through MyD88 and MyD88-independent pathways, activating nuclear factor *kappa beta* (NF κ B) and resulting in the accumulation of chemokines and cytokines (O'Neill, 2006), we examined if abnormalities of TLRs are associated with depression and/or suicide. For that purpose we determined the protein and mRNA expression in dorsolateral prefrontal cortex (DLPFC) obtained from depressed suicide victims, non-depressed suicide victims, depressed non-suicide subjects, and normal controls. Of all the TLRs, we specifically determined the gene and protein expression of TLR3 and TLR4 in this study because (i) TLR3 is the only TLR present in human neurons (Lafon et al., 2006); (ii) it is associated with cognitive function in mice (Okun et al., 2010) and; (iii) both TLR3 and TLR4 are involved in neurite growth and other neuronal functions (Okun et al., 2009). Both TLR3 and TLR4 are also unique compared to other TLRs because of their ability to activate interferon regulatory factor through the MYD88-independent signaling pathway (Okun et al., 2009).

2. Materials and methods

2.1 Subjects and diagnoses

The study was performed in the DLPFC (Brodmann area 9 [BA9]) of 22 depressed suicide victims, 11 non-depressed suicide victims, 12 depressed non-suicide subjects and 20 non-psychiatric control subjects, hereafter referred to as normal control subjects. Brain tissues were obtained from the Maryland Brain Collection at the Maryland Psychiatric Research Center, Baltimore, Maryland. Tissues were collected only after a family member gave informed consent. All tissue from normal control and suicide subjects was grossly examined by experienced neuropathologists. Toxicology data were obtained by the analysis of urine and blood samples. All procedures were approved by the University of Maryland Institutional Review Board (IRB) and by the University of Illinois IRB.

2.2 Diagnostic method

Subject diagnosis was based on the Structured Clinical Interview for DSM-IV (SCID) (Spitzer et al., 1992). At least one family member and/or a friend, after giving written informed consent, underwent an interview. Diagnoses were made by a consensus of two psychiatrists from the data obtained in this interview, medical records from the case, and records obtained from the Medical Examiner's office. Normal control subjects were verified as free from mental illnesses using these consensus diagnostic procedures.

2.3 Determination of mRNA levels

2.3.1 RNA isolation—Total RNA was extracted from 100 mg of tissue using the TRIZOL reagent according to the manufacturer's instructions and treated with DNase 1 (Invitrogen, USA). The RNA yield was determined by absorbance at 260 nm using NanoDrop®ND-1000 (NanoDrop Technologies, Montchanin, DE, USA). RNA quality was assessed using Agilent Bioanalyzer 2100. All samples had 28S/18S ratios >1.2 and RNA integrity number (RIN) above 6.6. The mean RIN was 7.2 ± 0.6 .

2.3.2 mRNA quantitation—Expression levels of mRNA were determined using a two-step real-time RT-PCR (qPCR) method, which we have previously published (Pandey et al., 2012). Briefly, 1 µg of total RNA was reverse transcribed using MMLV-reverse transcriptase (life technologies) in a final reaction volume of 20 µl. qRT-PCR was performed using pre-designed Taqman gene expression assays (Applied Biosystems, Foster City, CA) targeting TLR3, Hs 01551078_m1 and TLR4, Hs 00152939_m1 along with two housekeeping genes β -actin (ACTB), Hs99999903_m1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), Hs99999905_m1. For each primer/probe set, qPCR reaction is carried out using 10 µl of cDNA (diluted 1:10) in 1X TaqMan Universal PCR Master Mix (Applied Biosystems) as per manufacturer's instructions. Each qPCR plate included a "no reverse transcriptase" and "no template" control to eliminate non-specific amplification and each sample is assayed in triplicate.

For qPCR gene expression analysis, raw expression data (C_t) are normalised to the geometric mean of the two housekeeping genes. Outliers were excluded if the normalized (delta C_t) values were greater than 2 standard deviations from the group mean. Relative

expression levels, reported as fold change, were determined by $2^{-(\Delta C_t)}$ method, as described in Applied Biosystems User Bulletin No. 2 (P/N 4303859) and C_t values are used for further statistical analysis.

2.4 Determination of protein expression of TLRs by Western blot

Since TLRs are primarily present in the membrane fraction, the protein expression of TLRs was determined by the Western blot method in membrane fractions, as described by us earlier (Dwivedi & Pandey, 2000).

2.4.1 Quantitation of TLR3 and TLR4 in membrane fraction by Western blot—

Immunolabeling of TLR3 and TLR4 was determined as described in a previous study (Dwivedi et al., 2000). Briefly, equal volumes of membrane fractions isolated by this procedure (30 µg of protein in 20 µL) were loaded onto 7.5% (weight per volume) polyacrylamide gel and electrophoresed. The blots were initially developed using monoclonal anti-TLR3 (1:3000 dilution) and polyclonal anti-TLR4 antibody (1:3000 dilution) and subsequently using β-actin monoclonal antibody (1:5000 dilution). The levels of TLR3 and TLR4 proteins were calculated as a ratio of the optical density of the TLR antibody of interest to the optical density of β-actin antibody. TLR3 and TLR4 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, California, USA), and had molecular weights of 117 kDa and 95 kDa, respectively.

2.5 Statistical analysis and effect of confounding variables

We analyzed the data using SAS 9.2 statistical software package. We used ANCOVA (Proc GLM) to jointly compare control subjects, depressed suicide victims, non-depressed suicide victims, and depressed non-suicide subjects adjusting the effects of age, postmortem interval (PMI), and brain pH. Next, we examined the effect of each predictor on the TLR protein and mRNA expressions controlling for other predictors. For example, when the effect of age was examined on TLR (protein or mRNA expressions), we controlled other predictors such as PMI, brain pH, and the group effect. In addition, for multiple comparisons we used t-test with Bonferroni Correction to adjust the type I error rates. We also performed a post-hoc t-test for each paired comparison separately.

3. Results

3.1 Effect of Confounding Variables and Age on TLR3 and TLR4 Expression

The demographic and the clinical characteristics of the suicide victims, depressed subjects, and the normal control subjects are given in Table 1. Our ANCOVA analysis showed that only age has a significant inverse effect on protein or mRNA expression of TLR4 ($F=4.32$, $P=.04$) suggesting that increasing age is associated with decreased TLR4 expression. PMI and brain pH have no significant effects on protein or mRNA expressions of TLR3 and TLR4.

3.2 Protein expression of TLR3 and TLR4 in the DLPFC of depressed non-suicide subjects, depressed suicide victims, non-depressed suicide victims, and normal controls

Representative immunoblots of two normal controls, two depressed non-suicide subjects, two non-depressed suicide victims, and two depressed suicide victims are shown in the Figure 1. As can be seen, TLR3 and TLR4 protein expression appears to be increased in depressed suicide and non-depressed suicide victims compared with normal controls, but not in depressed non-suicide subjects. The mean TLR3 and TLR4 protein expressions of the normal control subjects, depressed suicide victims, non-depressed suicide victims, and depressed non-suicide subjects are shown in Figure 2A and B.

We compared the TLR3 and TLR4 expressions between the groups by performing ANCOVA. F-tests of ANCOVA showed that the above-mentioned four groups differ significantly in both TLR3 ($F=7.78$, $p=.0002$) and TLR4 ($F=3.52$, $p=.02$) protein expressions controlling for the other predictors. For multiple comparison using t-test with Bonferroni correction we found that there are significant increases in protein expressions of TLR3 and TLR4 in the depressed suicide victims and non-depressed suicides compared with normal control subjects.

For paired comparisons, we used post-hoc t-test. Our analysis showed that there are significant increases in protein expressions of TLR3 (t-test: depressed suicides vs. controls, $p=.0003$; non-depressed suicides vs. controls, $p=.03$) and TLR4 (t-test: depressed suicides vs. control, $p=.004$; non-depressed suicides vs. control, $p=.004$) in the depressed suicide victims and non-depressed suicides compared with normal control subjects. In contrast, the difference in protein expression of TLR3 (t-test: depressed non-suicides vs. controls, $p=.83$) and TLR4 (t-test: depressed non-suicides vs. controls, $p=.68$) between normal control subjects and the depressed non-suicide group was not statistically significant. These data thus suggest an increase in TLR3 and TLR4 protein expressions in depressed and non-depressed suicide victims, but not in depressed non-suicide subjects, when compared with normal controls.

3.3 Gene expression of the TLR3 and TLR4 in the DLPFC of suicide victims, depressed subjects, and normal controls

Since we observed an increase in TLR3 and TLR4 protein expressions in the DLPFC of the depressed suicide victims, we then examined if these changes in depressed suicide victims are also accompanied with increased TLR3 and TLR4 gene expression. The mean mRNA expression for TLR3 and TLR4 are shown in Fig. 3A & B.

The overall group effect using ANCOVA model for both TLR3 and TLR4 gene expression was significant (TLR3: $F=3.71$, $p=.02$; TLR4: $F=3.12$, $p=.03$) and suggests that the four groups differ significantly in both TLR3 and TLR4 gene expressions. For multiple comparisons using t-test with Bonferroni correction we found that there are significant increases in gene expressions of TLR3 in the depressed suicide victims, non-depressed suicides and depressed non-suicide subjects compared with normal control subjects. However, there are significant increases in gene expressions of TLR4 in the depressed

suicide victims, non-depressed suicides compared with normal control subjects but not in depressed non-suicide subjects.

For paired comparisons, we used post hoc t-test. We found that the mean mRNA expression of TLR3 (t-test: depressed suicide vs. controls, $p < .0001$) and TLR4 (t-test: depressed suicide vs. controls, $p < .0001$) in depressed suicide victims was significantly higher compared with normal control subjects (Figure 3A and B). Similarly, we found significant increases in mRNA expression levels of TLR3 and TLR4 for depressed non-suicide subjects and non-depressed suicide victims compared with normal controls. These data, thus, suggest an increase in TLR3 and TLR4 mRNA expression in depressed suicide victims, non-depressed suicide victims, and depressed non-suicide subjects compared with normal control subjects.

3.4 Relationship between TLR3 and TLR4 protein and mRNA expression

Since we found an increase in both protein and mRNA expression in the depressed suicide group, we examined if there was one-to-one correlation between mRNA and protein expression of TLR3 and TLR4 in the total sample and in individual groups. We did not find significant correlation between TLR3 and TLR4 mRNA and protein expression either in the total sample or in any of the groups.

3.5 Effect of suicide method, antidepressant medication, and ethanol on TLR3 and TLR4 expression

a. Suicide Method: We examined the effect of the method of suicide on the protein and the mRNA expression of TLR3 and TLR4 in the DLPFC of depressed suicide victims, non-depressed suicide victims, and depressed non-suicide subjects. We did not find any significant effects of the method of suicide between those depressed suicide victims who died by violent means ($n = 11$) or non-violent means ($n = 11$), such as drug overdose. This was also the case when we compared the violent means ($n = 9$) and non-violent means ($n = 2$) in the non-depressed suicide group.

b. Effects of antidepressants and ethanol: Since several of the subjects in the depressed suicide group and the majority of the subjects in the non-suicide depressed group were on antidepressant medication at the time of death, we examined if the presence of antidepressants had any influence on the protein and/or mRNA expression of TLR3 and TLR4. In the suicide group, there were 10 subjects who were on different types of antidepressants and 14 subjects who were free of any psychotropic medication. In the non-suicide depressed group, 10 out of 12 subjects were on antidepressant medication at the time of death. When we compared the protein and mRNA expression of TLR3 and TLR4 between the depressed suicide subjects who were on antidepressants with those who were free of any medication, we did not find any significant differences in the protein and mRNA expression of TLR3 or TLR4 between these two groups, suggesting that the presence of antidepressants does not have a significant influence on the expression of TLR3 and TLR4. Since in this group we did not find any significant effect of antidepressant treatment, it is quite likely that the effect of antidepressants on TLR3 and TLR4 expression in the non-suicide depressed group is non-significant. Thus, the differences in the TLR3 and TLR4

expression in the depressed suicide group and non-suicide depressed group compared with normal controls do not appear to be related to the effect of antidepressant medication.

We also examined the effect of ethanol in the non-depressed suicide group. Out of eleven subjects, 5 showed the presence of ethanol and/or cocaine and 6 had no presence of ethanol or cocaine. When we compared TLR3 and TLR4 mRNA and protein expression between these groups, we found that there was no significant difference in the protein and mRNA expression of TLR3 and TLR4 in the non-depressed suicide group, between those who have shown the presence of ethanol and those who did not have the presence of ethanol. Thus, it appears that the presence of ethanol and/or cocaine does not have any significant influence on the expression of TLR3 and TLR4.

1. Discussion

In this study we found that the mRNA levels of TLR3 and TLR4 are increased in all three groups (i.e., depressed suicide, non-depressed suicide and depressed non-suicide) compared with controls. We also found that the protein expression of TLR3 and TLR4 was significantly increased in both depressed and non-depressed suicide victims, but not in depressed non-suicide subjects compared with normal controls. Thus, the protein and mRNA studies of TLR3 and TLR4 showed a dissociation in depressed non-suicide subjects, but not in suicide victims (either depressed or non-depressed). The reason for this dissociation of mRNA and protein expression levels of TLR3 and TLR4 in depressed non-suicide subjects is unclear.

The main objective of the present investigation was to examine if abnormalities in TLR3 and TLR4 expression were associated with suicide. Since we found that TLR3 and TLR4 protein and mRNA expression was increased in DLPFC of depressed suicide subjects, we then examined if these abnormalities are specific to suicide with depression or are shared by suicide victims with other diagnoses. To address this issue, we then determined the TLR3 and TLR4 expression in the DLPFC of non-depressed suicide victims, with diagnoses other than depression. We found that in the non-depressed suicide group the protein and mRNA expression of TLR3 and TLR4 was significantly increased compared with controls, an observation similar to that in depressed suicide victims.

In order to examine if abnormalities of TLR3 and TLR4 are also associated with depression independent of suicide, we determined TLR3 and TLR4 expression in non-suicide depressed subjects. We found that the mRNA expression of the TLR3 and TLR4 is increased in the DLPFC of depressed non-suicide subjects, but there were no significant differences in TLR3 and TLR4 protein expression in this group compared with controls. These results suggest that TLR3 and TLR4 expression may be dysregulated in suicide, independent of diagnoses, and that TLR3 and TLR4 mRNA but not protein expression is dysregulated in depressed non-suicidal subjects.

Although the reasons for dissociation between TLR3 and TLR4 protein and mRNA expression in depressed non-suicide subjects are unclear, a similar dissociation was reported by Bosisio et al. (2002) for TLR4 receptors activated by lipopolysaccharide (LPS). They found that LPS has divergent effects on TLR4 expression at the mRNA and protein levels

with upregulation of mRNA and downregulation of protein. Our observation of increased mRNA but not protein in depressed non-suicide is similar to this observation.

The innate immune response is the first line of defense against infectious disease (Hanke et al., 2011). The multitude of pathogens is effectively detected by pattern recognition receptors (PRRs) that recognize specific and typical pathogen-associated molecular patterns (PAMP) (Kawai & Akira, 2007), and danger signals (danger-associated molecular patterns [DAMP])(Kawai et al., 2007; Klune et al., 2008; Piccinini & Midwood, 2010), and initiate a defensive immune response. TLRs constitute a phylogenetically conserved family of PRRs that recognize multiple PAMPs, and DAMPs (Piccinini et al., 2010) and regulate the activation of both innate and adaptive immunity (Hoebe & Beutler, 2004; Qureshi & Medzhitov, 2003). As the CNS mounts an organized innate immune response not only during infection, but also in response to neuronal injury (Klune et al., 2008), TLRs are a key link between the innate immune system and the CNS (Crack et al., 2007; Hanke et al., 2011; Okun et al., 2011). In general, pathogens, injury, and stressors stimulate TLRs on microglia or other cells, recruit adaptor proteins, and initiate the proinflammatory signal transduction pathways that ultimately trigger cytokine transcription (Hanke et al., 2011; Okun et al., 2009).

TLRs are a family of innate immune receptors (Kawai et al., 2007), and the first TLR protein was discovered in *Drosophila melanogaster* (Valanne et al., 2011). Subsequently, many additional TLRs have been identified across diverse species including the human brain (Bsibsi et al., 2006; Lafon et al., 2006). Recent studies suggest that mammalian TLRs possess developmental roles during embryogenesis (Ma et al., 2006), as well as physiological and metabolic roles in adults (Okun et al., 2011; Rolls et al., 2007). TLRs are expressed in a variety of mammalian immune system related cell types, but they have also been shown to be widely expressed in the CNS, and many of these TLRs have also been reported to be expressed in the human brain (Bsibsi et al., 2002; Lafon et al., 2006). About 11 different TLRs have been identified in humans, and among these, TLR3 and TLR4 appear to be of interest in CNS innate immunity and also in neuronal function (Okun et al., 2011).

TLR4 has been implicated in the pathogenesis of cognitive impairment, alcoholism (Crews et al., 2013), as well as in AD (Tahara et al., 2006). TLR3 is probably the only TLR present in the human neuron (Bsibsi et al., 2006) and has significant effects on cognitive performance of mice in hippocampal dependent and independent tasks (Okun et al., 2010). A deficiency of TLR3 confirms superior performance in spatial working memory and cognition (Okun et al., 2011). TLR3 affects both hippocampal-dependent and independent behavior. Adult TLR3 KO mice exhibit enhanced hippocampal-dependent working memory in a Morris maze novel object recognition and contextual fear conditioning tasks (Okun et al., 2010). Intracerebroventricular injection of TLR3 ligand impairs working memory (Hanke et al., 2011). In a recent study, Crews et al. (Crews et al., 2013) reported that the protein expression of TLR2, TLR3 and TLR4 was significantly increased in the orbitofrontal cortex of alcoholic subjects compared with controls. This increase in alcoholic brain appears to be similar to our observation in suicide. These observations suggest that TLR3 and TLR4 may play an important role in psychiatric disorders.

TLR3 is the only TLR that does not signal through MYD88, but signals via TRIF recruitment, causing the activation of the transcription factor IRF-3, leading to the expression of IFN- β and IFN- α (Marsh et al., 2009). Activation of TRIF can also cause its interaction with TRAF-6, which, along with RPI, mediates NF κ B activation, resulting in the production of cytokines and chemokines. TLR3 is thus involved in the production of IFN- β as well as cytokines [see Okun et al. (2009)].

Different TLRs may have different physiologic functions and recognize specific PAMPS or DAMPS [for review, see Guo and Schluesener (2007)]. For example, lipopolysaccharide (LPS) is recognized by TLR2 (Kielian, 2006) and TLR4. Lipoproteins are recognized by TLR2 and TLR1 or TLR6, and dsRNA is recognized by TLR3 (Alexopoulou et al., 2001; Kielian, 2006; Takeda & Akira, 2005). The known endogenous ligands for TLRs are either molecules released from damaged cells or extracellular matrix breakdown products (Guo et al., 2007).

The expression of TLRs is regulated by many factors such as cytokines, PAMPS and DAMPS (microbial viruses). LPS has been shown to regulate TLR4 expression and it has been shown by Bosisio et al. (2002) that LPS increases TLR4 mRNA levels but concomitantly decrease its surface expression. They reported that IFN- γ counteracted the LPS-induced downregulation of TLR4. Viral infection induces the expression of TLR3 mRNA in macrophages and this increased expression is suppressed by treatment with anti-IFN- α/β antibody, indicating that IFN- α/β modulates virus-induced TLR3 expression (Miettinen et al., 2001; Takeda et al., 2003). Thus, it is quite possible that the observed increase in TLR3 and TLR4 may be related to PAMPS or DAMPS resulting in LPS production, as well as increased levels of cytokines.

Our observation that protein expression of TLR3 and TLR4 were significantly increased in DLPFC of depressed and non-depressed suicide victims, but not in depressed non-suicide subjects suggests that TLR3 and TLR4 protein expression is dysregulated in suicide independent of diagnosis, but probably not in depression. This was somewhat surprising since the abnormalities of cytokines protein levels have been reported both in depression and in suicide (Pandey et al., 2012; Schiepers et al., 2005; Steiner et al., 2008). However, there are some studies that suggest that cytokine abnormalities may differ between suicidal and non-suicidal patients. For example, Mendlovic et al. (1999) found decreased IL-4 and IL-5 (anti-inflammatory cytokines) plasma levels in suicidal patients with MDD compared to non-suicidal patients with MDD. Janelidze et al. (2011) found increased levels of IL-6 and TNF- α and decreased levels of IL-2 in the plasma of suicidal MDD patients compared with non-suicidal MDD patients.

In conclusion, this is the first study, to our knowledge, of TLRs in the postmortem brain of psychiatric and/or suicidal subjects; although a role of TLRs in Alzheimer's disease has been suggested, based on a mouse model (Letiembre et al., 2009) and postmortem brain studies of Alzheimer's patients (Landreth & Reed-Geaghan, 2009) and postmortem brain of alcoholic subjects (Crews et al., 2013). Since different TLRs are involved in different functions, it will be important to examine the role of other TLRs in the postmortem brain of psychiatric and suicidal subjects.

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Figure 1.

Representative Western blots showing the immunolabeling of TLR3, TLR4, and β -actin in the DLPFC membrane fractions of two normal controls, two depressed non-suicide subjects, two non-depressed suicide victims, and two depressed suicide victims.

kDa indicates kilo Daltons.

Figure 2.

Mean protein expression levels of TLR3 (A) and TLR4 (B) in the DLPFC membrane fractions of normal controls (n = 20), depressed suicide victims (n = 22), non-depressed suicide victims (n = 11), and depressed non-suicide subjects (n = 12). The results are expressed as optical density (O.D.). Values are mean $\pm$ SD. *p < .05

Figure 3. Mean mRNA expression levels of TLR3 and TLR4 in the DLPFC

(A) The mean mRNA expression levels of TLR3 in the DLPFC of normal controls 1.00 ± 0.09 ($n = 20$), depressed suicide victims 1.26 ± 0.06 ($n = 22$), depressed non-suicide subjects 1.37 ± 0.10 ($n = 12$), and non-depressed suicide victims 1.35 ± 0.10 ($n = 11$).

(B) The mean mRNA expression levels of TLR4 in the DLPFC of normal controls 1.00 ± 0.09 ($n = 20$), depressed suicide victims 1.29 ± 0.09 ($n = 22$), depressed non-suicide subjects 1.35 ± 0.12 ($n = 12$), and non-depressed suicide victims 1.32 ± 0.11 ($n = 11$). The data are shown as fold change in mRNA levels. Values are fold change $\pm$ S.E.M.

* $p < .05$

Table 1

Demographic Characteristics of Suicide, Depressed, and Normal Control Subjects

		Age (years)	Race	Gender	PMI (hours)	Brain pH	Cause of Death	Psychotropic drugs (at the time of death)	Psychiatric Diagnosis
Normal Control Subjects^a									
1.	CONTROL	22	Black	Male	19	6.9	GSW	None	Normal
2.	CONTROL	42	White	Female	23	7.2	Pneumonia	None	Normal
3.	CONTROL	37	Black	Male	5	7.1	ASCVD	None	Normal
4.	CONTROL	31	Black	Male	8	7.2	GSW	None	Normal
5.	CONTROL	46	Black	Male	9	7.1	Multiple injuries	None	Normal
6.	CONTROL	33	White	Male	15	7.0	GSW	None	Normal
7.	CONTROL	48	White	Male	26	6.9	ASCVD	None	Normal
8.	CONTROL	40	White	Female	7	7.0	ASCVD	None	Normal
9.	CONTROL	23	Black	Male	15	6.8	GSW	None	Normal
10.	CONTROL	65	Black	Female	23	6.9	ASCVD	None	Normal
11.	CONTROL	35	White	Male	24	6.9	Crush injury to abdomen and chest	None	Normal
12.	CONTROL	37	White	Male	24	7.0	ASCVD	None	Normal
13.	CONTROL	45	White	Male	22	7.3	ASCVD	None	Normal
14.	CONTROL	26	White	Male	12	6.9	Arrhythmia	None	Normal
15.	CONTROL	47	White	Male	10	7.0	ASCVD	None	Normal
16.	CONTROL	31	White	Male	16	7.2	MVA	None	Normal
17.	CONTROL	60	White	Male	15	7.1	Accidental drowning	None	Normal
18.	CONTROL	28	White	Male	13	6.8	Electrocution	None	Normal
19.	CONTROL	45	White	Female	16	6.9	Cardiac arrhythmia	None	Normal
20.	CONTROL	62	White	Male	19	7.0	Cardiac arrest	None	Normal
Depressed Suicide Victims^b									
1.	SUICIDE	27	White	Male	24	7.0	GSW	None	MDD, Ethanol abuse
2.	SUICIDE	44	White	Female	11	7.2	Drug OD	Nortriptyline	MDD, Ethanol abuse
3.	SUICIDE	36	White	Female	10	7.1	GSW	None	MDD
4.	SUICIDE	24	White	Male	7	7.1	GSW	Ethanol	MDD

	Age (years)	Race	Gender	PMI (hours)	Brain pH	Cause of Death	Psychotropic drugs (at the time of death)	Psychiatric Diagnosis
5.	SUICIDE	White	Male	12	7.0	Drug OD	None	MDD, Polysubstance Abuse
6.	SUICIDE	White	Male	23	6.9	Jump from height	None	MDD
7.	SUICIDE	White	Female	27	7.1	Drug OD	Amitriptyline, Desipramine, Nortriptyline, Ethanol	MDD, Ethanol abuse
8.	SUICIDE	Black	Female	16	7.3	Drug OD	None	MDD
9.	SUICIDE	White	Female	21	6.9	Drug OD	Amitriptyline, Desipramine, Ethanol	MDD
10.	SUICIDE	White	Female	18	7.2	GSW	None	MDD
11.	SUICIDE	White	Male	24	7.0	Drug OD, Ethanol intoxication	Ethanol	MDD, Ethanol abuse
12.	SUICIDE	White	Female	16	6.8	Drug OD	Nortriptyline	MDD, Panic disorder with agoraphobia
13.	SUICIDE	White	Male	12	7.0	Hanging	Paroxetine	MDD
14.	SUICIDE	White	Male	17	7.1	Hanging	Venlafaxine	MDD
15.	SUICIDE	White	Female	30	7.2	Drug OD, Ethanol intoxication	Fluoxetine, Ethanol	MDD, Ethanol abuse, Opioid abuse
16.	SUICIDE	White	Female	27	7.0	Venlafaxine OD	Venlafaxine, Ethanol	MDD, Ethanol abuse
17.	SUICIDE	White	Male	14	6.8	Hanging	Ethanol	MDD
18.	SUICIDE	Black	Male	23	6.9	Hanging	None	MDD
19.	SUICIDE	White	Male	19	6.9	Drug OD, Ethanol intoxication	Ethanol	MDD
20.	SUICIDE	White	Male	22	7.0	GSW	Fluoxetine, Venlafaxine	MDD
21.	SUICIDE	White	Female	20	7.0	Drug OD	Xanax	MDD
22.	SUICIDE	White	Male	26	7.1	Stabbing	Sertraline	MDD
Depressed Non-Suicide Subjects^c								
1.	Depression Non-Suicide	White	Male	14	6.9	ASCVD	None	MDD
2.	Depression Non-Suicide	Black	Female	8	6.4	ASCVD	Fluoxetine, Ethanol	MDD, Polysubstance abuse
3.	Depression Non-Suicide	White	Male	4	6.3	ASCVD	Bupropion	MDD, Cerebral palsy
4.	Depression Non-Suicide	Black	Female	7	6.7	ASCVD	Paroxetine Thioridazine	MDD
5.	Depression Non-Suicide	White	Male	11	7.0	MVA	Sertraline	MDD, Polysubstance abuse
6.	Depression Non-Suicide	White	Male	36	6.8	Fatty Liver	Thioridazine	MDD with psychosis
7.	Depression Non-Suicide	Black	Male	20	7.1	Seizure disorder	Fluoxetine, Risperidone	MDD with psychosis

		Age (years)	Race	Gender	PMI (hours)	Brain pH	Cause of Death	Psychotropic drugs (at the time of death)	Psychiatric Diagnosis
8.	Depression Non-Suicide	59	White	Male	20	7.0	ASCVD	Sertraline	MDD, Ethanol abuse
9.	Depression Non-Suicide	46	White	Female	23	6.9	Mixed drug intoxication	Bupropion, Lamotrigine	MDD, Ethanol abuse, Polysubstance abuse
10.	Depression Non-Suicide	29	White	Female	22	6.9	Morbid obesity, Cardiomegaly	Fluoxetine, Norfluoxetine, Norpropoxyphene	MDD
11.	Depression Non-Suicide	49	White	Male	24	7.1	ASCVD	Desmethyl-sertraline	MDD
12.	Depression Non-Suicide	47	White	Female	26	6.5	DKA	Fluoxetine	MDD
Non-Depressed Suicide Victims^d									
1.	SUICIDE	34	White	Male	16	6.4	GSW	Ethanol	Ethanol abuse
2.	SUICIDE	21	White	Male	17	6.8	GSW	None	Adjustment disorder mixed
3.	SUICIDE	75	White	Male	18	6.4	GSW	None	Adjustment/conduct disorder
4.	SUICIDE	87	White	Male	16	6.6	GSW	None	Adjustment/conduct disorder
5.	SUICIDE	39	White	Male	30	6.7	Asphyxia	Freon, cocaine and metabolites	Cocaine abuse
6.	SUICIDE	30	White	Male	32	6.5	Hanging	Cocaine, Ethanol	Ethanol abuse, Cocaine abuse, Drug abuse
7.	SUICIDE	40	White	Male	26	6.7	GSW	Ethanol	Adjustment disorder
8.	SUICIDE	20	White	Male	32	6.9	Hanging	Ethanol	Ethanol abuse
9.	SUICIDE	71	White	Female	24	6.5	Drug OD	None	Adjustment disorder mixed
10.	SUICIDE	24	White	Male	22	6.8	Hanging	None	Schizoaffective disorder
11.	SUICIDE	21	White	Male	23	6.8	Hanging	None	Adjustment/conduct disorder

Abbreviations: ASCVD, atherosclerotic cardiovascular disease; CO, carbon monoxide; DKA, diabetic ketoacidosis; GSW, gunshot wound; MDD, major depressive disorder; MVA, motor vehicle accident; OD, overdose; PMI, postmortem interval

^aMean $\pm$ SD age is 40.15 $\pm$ 12.39 years; PMI, 16.05 $\pm$ 6.32 h; and brain pH 7.01 $\pm$ 0.14

^bMean $\pm$ SD age is 40.82 $\pm$ 14.70 years; PMI, 19.05 $\pm$ 6.28 h; and brain pH 7.03 $\pm$ 0.13

^cMean $\pm$ SD age is 49.50 $\pm$ 17.18 years; PMI, 17.92 $\pm$ 9.32 h; and brain pH 6.80 $\pm$ 0.27

^dMean $\pm$ SD age is 42.00 $\pm$ 24.22 years; PMI, 23.27 $\pm$ 6.16 h; and brain pH 6.65 $\pm$ 0.18