(Fig. 3D). These results suggest that minocycline protects against oligodendrocyte degeneration through inhibiting at least the production of TNF-α from microglial cells as well as aripiprazole.

3.4. Aripiprazole and minocycline inhibit phosphorylation of STAT1

Previous reports have suggested that increased transcription of TNF-α mRNA by IFN-γ uses the JAK-STAT1 pathway (Takagi et al., 2005). To determine whether minocycline or aripiprazole inhibit an IFN-γ-mediated increase in the expression of pSTAT1, we performed immunocytochemistry for pSTAT1 in these drugs-pretreated HAPI cells. pSTAT1 was strongly stained in the nucleus of positive control cells (IFN-γ-treated group) compared to control cells. In contrast, inhibition of phosphorylated STAT1 was observed in minocycline- or aripiprazole-pretreated cells (Fig. 4A). Additionally, the intensity of pSTAT1 was significantly attenuated to control levels with pretreatment by minocycline or aripiprazole (Fig. 4B).

4. Discussion

This is the first report to demonstrate that pretreatment of aripiprazole and minocycline inhibits oligodendrocyte damage by suppressing IFN-γ-activated microglial cells.

We have already demonstrated that aripiprazole inhibits TNF-α production from IFN-γ-activated microglial cells via suppressing the elevation of intracellular calcium signaling, not via D2R receptor-related signaling (Kato et al., 2008). In the present study, we have proved that aripiprazole and minocycline have the potential to inhibit phosphorylation of STAT1 and lead to the attenuation of TNF-α production in microglial cells. The JAK1/STAT1 pathway is activated by IFN-γ and is an essential regulator of diverse cellular functions including cytokine production (Gough et al., 2008). The plasma concentration of IFN-γ was reported to be significantly higher in patients with schizophrenia than in controls (Kim et al., 2004), and minocycline has been reported to be effective in the treatment of schizophrenia (Ahuja and Carroll, 2007; Miyaoka et al., 2007; Miyaoka, 2008; Chaves et al., 2010; Levkovitz et al., 2010; Kelly et al., 2011; Chaudhry et al., 2012). Together, it is suggested that abnormal constitutive activation of JAK1-STAT1 pathway may be related to the pathophysiology of schizophrenia. Further investigations are required regarding these molecules.

The primary target of minocycline and aripiprazole on microglial cells has not been well clarified. Giuliani et al. have shown the direct action of minocycline on activated T cells and on microglia, which resulted in the decreased ability of T cells to contact microglia (Giuliani et al., 2005). Nikodemova et al. have shown that the inhibitory effect of minocycline on microglial activation is stimulus-specific and that it also downregulates MHC class II expression in microglia (Nikodemova et al., 2007). Horvath et al. have shown the inhibitory effect of minocycline on microglial migration (Horvath et al., 2008). Further evidence of other cytokine pathways interacting with minocycline is required to confirm the precise target of minocycline with a particular focus on microglia. The expression of TNF-α mRNA has been reported to be ablated by inhibiting phosphorylation of ERK and p38 (Rutault et al., 2001; Fan et al., 2007). Furthermore, it has been reported that minocycline attenuates the phosphorylation both of P38 and ERK (Nikodemova et al., 2006), and also we confirmed the same results (data not shown). This may indicate that the suppression of phosphorylated ERK and p38 by minocycline results in the attenuation of TNF-α release from activated HAPI microglial cells.

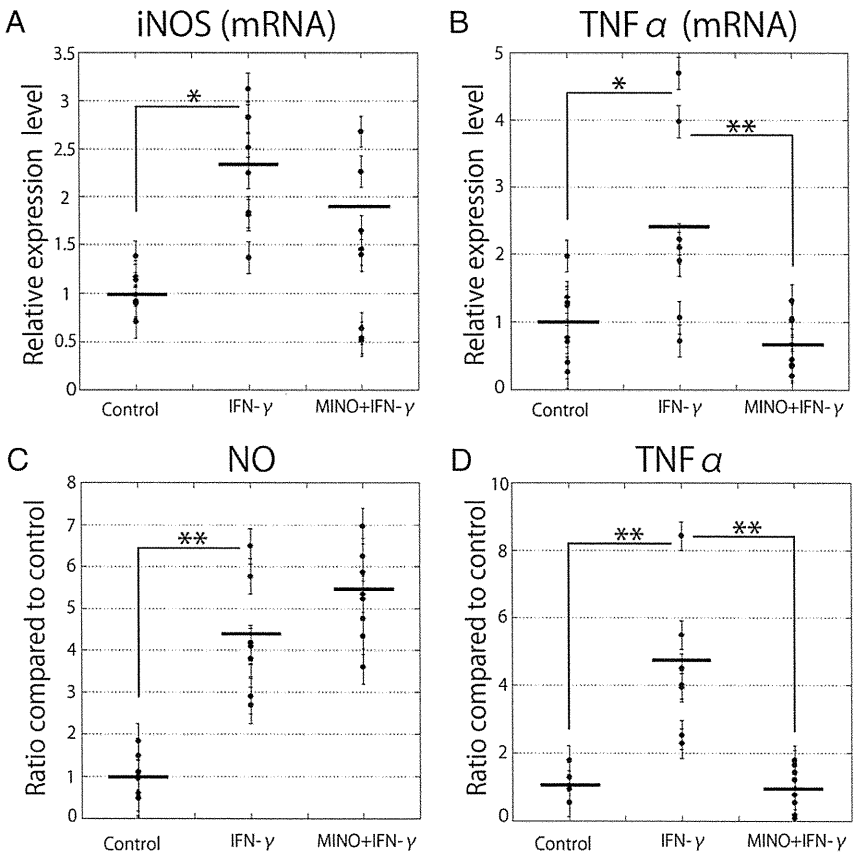

Fig. 3. Anti-inflammatory effects of minocycline on microglial cells. Real time PCR results showed that the relative expression of iNOS was upregulated by IFN-γ stimulation, minocycline did not inhibit the induction of iNOS expression (A). TNF-α was induced by IFN-γ-stimulation at mRNA level, and minocycline significantly attenuated the expression of TNF-α (B). NO release was induced by IFN-γ in HAPI cells, and minocycline did not inhibit NO release (C). Minocycline significantly inhibited TNF-α release by IFN-γ-activated HAPI cells (D). Each result was shown as the mean ± SEM (*$p < 0.05$; **$p < 0.01$ in comparison to the IFN-γ or control group). Abbreviations in A, B, C and D: MINO, minocycline.

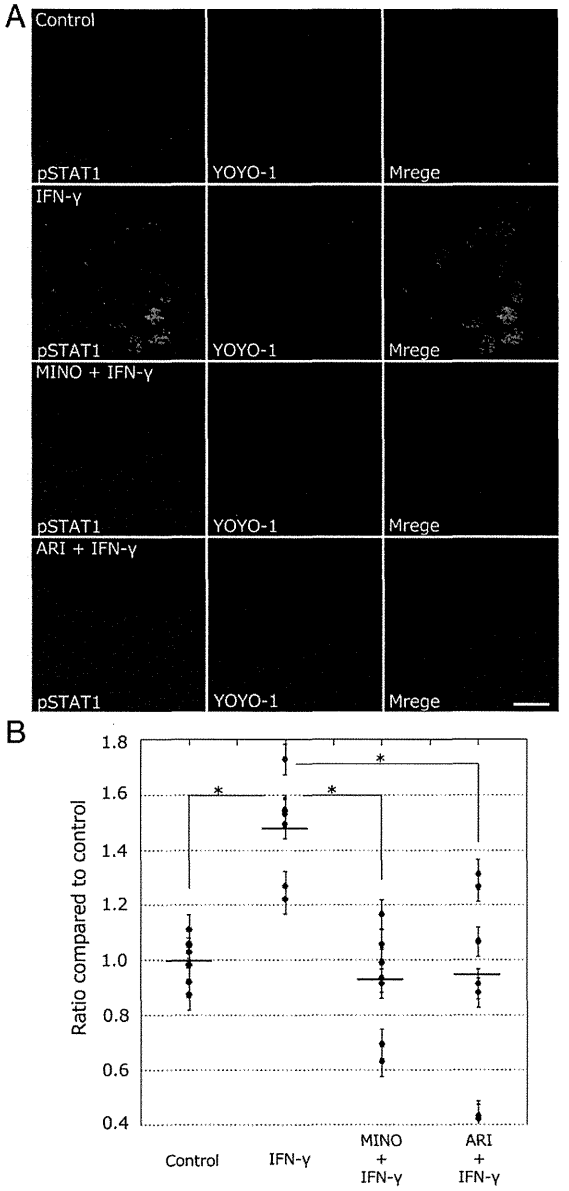

Fig. 4. Immunofluorescence for pSTAT1. pSTAT1 immunoreactivity in the presence or absence of aripiprazole or minocycline followed by the addition of IFN-γ (A). pSTAT1 intensity is significantly attenuated in MINO- or ARI-pretreated cells compared to IFN-γ-treated HAPI cells (B). Each result was shown as the mean ± SEM (#$p < 0.05$ in comparison to the control group. *$p < 0.05$ in comparison to the IFN-γ or control group). Scale bar = 20 μm. Abbreviations in A and B: MINO, minocycline; ARI, aripiprazole.

Aripiprazole may have similar intracellular mechanisms as reported in a previous in vitro study (Kato et al., 2008). Further investigations should be conducted to clarify deeper underlying molecular mechanisms.

Traditionally, most studies on schizophrenia have focused on alterations in gray matter, however, recent evidence suggests that neurodevelopmental problems also occur in white matter, especially in oligodendrocytes and in the myelin that these cells produce. Xiao et al. reported that demyelination in the brains of mice which had been administered a copper chelator, cuprizone, led to abnormal behaviors such as spatial working memory impairment and hyperlocomotion (Xiao et al., 2008). Because the former is regarded as an endophenotype of schizophrenia, they proposed that cuprizone-fed mice may represent an animal model of schizophrenia. This study and their following study (Zhang et al., 2008) also revealed that quetiapine, an atypical antipsychotic, ameliorated both the demyelination and the abnormal behaviors, underscoring the causal relationship of the demyelination with symptoms of schizophrenia. In addition to the above reports, it has been reported that the numbers of CD68+, MHC class II+, and allograft inflammatory factor-1 (AIF-1)+ microglia, which are identified as microglial activation (Renaud et al., 2005), are higher in white matter than in gray matter (Mittelbronn et al., 2001). Therefore, microglial activation may cause more damage in white matter than in gray matter, and our in vitro co-culture method would help to reenact the in vivo mechanisms of schizophrenia by investigating interaction between microglia and oligodendrocytes. Recent MRI imaging studies suggest that antipsychotics impact on intracortical myelination in schizophrenia patients (Bartzokis et al., 2007, 2009, 2012). Bartzokis et al. have shown the protective properties of risperidone, an atypical antipsychotic, compared with fluphenazine, a typical antipsychotic, on intracortical myelination in schizophrenia (Bartzokis et al., 2009). When compared with the healthy controls and fluphenazine-treated schizophrenia patients, risperidone-treated schizophrenia patients showed significantly higher intracortical myelin volume. In the present study, we have not investigated the effects of risperidone, while our previous studies have shown that risperidone and quetiapine, but not haloperidol, inhibit IFN-γ-activated microglial activation in vitro (Kato et al., 2007; Bian et al., 2008). Therefore, based on the present study, we hypothesize that risperidone and quetiapine may have protective properties on intracortical myelination and white matter protection via suppressing microglial activation, and further in vitro and in vivo investigations should be conducted.

Kato et al. reported that aripiprazole inhibits the release of both NO and TNF-α from activated microglia, while haloperidol inhibits only NO release (Kato et al., 2007, 2008). Additionally, one recent report suggests that NO production does not appear to correlate with oligodendrocyte process retraction and cell death, while oligodendrocytes are highly sensitive to TNF-α (Li et al., 2008). Upregulation of TNF-α production has been reported in the prefrontal cortex of schizophrenia patients (Paterson et al., 2006). In our experiments, minocycline was found to inhibit at least TNF-α release from HAPI microglial cells and resulted in the protection of oligodendrocyte degeneration. In addition, aripiprazole was found to have a similar effect as minocycline on oligodendrocyte degeneration induced by microglial activation while in the haloperidol-pretreated group, oligodendrocytes exhibited focal bead-like swellings at their processes, and this change is one of the characteristics observed in oligodendrocyte degeneration (Li et al., 2008). These results thus suggest that minocycline as well as aripiprazole suppress oligodendrocyte degeneration through the inhibition of TNF-α release from activated microglia. Further studies are required to develop deeper understandings of inhibitory mechanisms of NO and TNF-α release utilizing other activators of microglia such as LPS and PMA. To investigate the direct influence of TNF-α, inhibitory experiments with TNF-α inhibitors are required. In addition, our previous report suggests that haloperidol does not suppress the release of IL-6 or IL-1β from interferon-γ-activated microglia (Kato et al., 2007). Therefore, in our co-culture experiment, these cytokines may also be differently affected by aripiprazole and haloperidol. Further experiments should be conducted.

On the other hand, one recent report suggests that minocycline has the potential to promote favor remyelination directly on oligodendrocytes (Defaux et al., 2011). In our experiments, only HAPI microglial cells were pre-treated with minocycline, but there is the possibility that a small amount of minocycline adhered to the tissue culture insert may be acting directly on oligodendrocytes. Further research is thus required to confirm the effects of minocycline and antipsychotics on oligodendrocyte remyelination directly or indirectly via microglial cells. Contrary to our results, Miller et al. have demonstrated that activated microglia increase survival and reduce apoptosis of mature oligodendrocytes (Miller et al., 2007). Their study used LPS for the stimulation of microglia instead of IFN-γ. This may explain the difference.

On the other hand, in our experimental design, the target drugs (aripiprazole, haloperidol and minocycline) were administered as pretreatment to IFN-γ activation, and not after IFN-γ challenge. Ideally, to make a therapeutic model of diseases (first inducing pathology, then intervening therapy), we should have added IFN-γ before the target drug treatment. However, IFN-γ challenge before the target drugs seems to induce significant oligodendrocyte damage in all the experimental conditions. Thus, in order to minimize oligodendrocyte damage due to pretreatment of IFN-γ challenge, we have chosen to treat the target drugs before IFN-γ challenge. While, in vivo it is expected that microglia activation will occur before the administration of minocycline and antipsychotics. Thus, the present experimental design may have not reflected actual process in vivo. Therefore, further detailed mechanisms should be investigated in the future in vivo experiments.

Recently, several studies have reported elevated S100B serum levels in schizophrenia (Steiner et al., 2009). S100B is synthesized in and released from astrocytes and oligodendrocytes, acting as a dose-dependent growth factor for neurons and glial cells (Steiner et al., 2008b). Nanomolar levels of S100B stimulate neurite growth and promote neuronal survival. However, micromolar levels of S100B result in opposite effects and can induce neuronal apoptosis (Van Eldik and Wainwright, 2003). In addition, Steiner et al. have reported that schizophrenia may be caused by white matter oligodendrocyte damage of dysfunction, associated with a release of S100B into body fluid (Steiner et al., 2008a). In our experiments, pretreatment of aripiprazole and minocycline inhibited oligodendrocyte damage by suppressing IFN-γ-activated microglial cells. Thus, S100B may be modulated by aripiprazole and/or minocycline in our co-culture model. Further studies are required.

Our results may lead to a deeper understanding of the pathophysiology of schizophrenia and establish novel therapeutic strategies for schizophrenia with anti-inflammatory/immunosuppressive agents. Further molecular mechanisms of the inhibitory effect of aripiprazole and minocycline on microglial activation should be clarified. In vivo studies to confirm the present results should also be performed using animal models of schizophrenia such as the cuprizone model.

Role of funding source

This work was financially supported by Grant-in-Aids from the Japan Society for the Promotion of Science (JSPS) [to YS, TAK, AM, YM and SK], and from the Mitsubishi Pharma Research Foundation [to TAK and HH].

Contributors

All authors contributed substantially to the scientific process leading up to the writing of the present paper. TAK and AM, the principal investigators of the present research, and YS, the first author, made the conception and design of the project and wrote the protocol. The performance of experiments and the data analysis/interpretation were done by YS, TAK, AM, YM, HH and MSK. YS wrote the first draft of the manuscript. Critical revisions of the manuscript were made by TAK, AM, DY and SK. All authors contributed to and have approved the final manuscript.

Conflict of interest

All authors declare that they have no financial conflict of interest.

Acknowledgments

The authors would like to thank Dr. Masahiro Ohgidani, Dr. Noriaki Sagata, Dr. Yusuke Yamauchi, and Dr. Kohei Hayakawa (Molecular & Translational Psychiatry Unit, Department of Neuropsychiatry, Kyushu University) and Dr. Daichi Muratsu (Division of Oral and Maxillofacial Oncology, Faculty of Dental Science, Kyushu University) for fruitful comments and technical supports.

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frontiers in
HUMAN NEUROSCIENCE

HYPOTHESIS AND THEORY ARTICLE
published: 04 February 2013
doi: 10.3389/fnhum.2013.00013

Are microglia minding us? Digging up the unconscious mind-brain relationship from a neuropsychoanalytic approach

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The unconscious mind-brain relationship remains unresolved. From the perspective of neuroscience, neuronal networks including synapses have been dominantly believed to play crucial roles in human mental activities, while glial contribution to mental activities has long been ignored. Recently, it has been suggested that microglia, glial cells with immunological/inflammatory functions, play important roles in psychiatric disorders. Newly revealed microglial roles, such as constant direct contact with synapses even in the normal brain, have defied the common traditional belief that microglia do not contribute to neuronal networks. Recent human neuroeconomic investigations with healthy volunteers using minocycline, an antibiotic with inhibitory effects on microglial activation, suggest that microglia may unconsciously modulate human social behaviors as "noise." We herein propose a novel unconscious mind structural system in the brain centering on microglia from a neuropsychoanalytic approach. At least to some extent, microglial activation in the brain may activate unconscious drives as "psychological immune memory/reaction" in the mind, and result in various emotions, traumatic reactions, psychiatric symptoms including suicidal behaviors, and (psychoanalytic) transference during interpersonal relationships. Microglia have the potential to bridge the huge gap between neuroscience, biological psychiatry, psychology and psychoanalysis as a key player to connect the conscious and the unconscious world.

Keywords: microglia, psychoanalysis, emotion, stress, unconscious, death instinct, suicide, psychiatry

INTRODUCTION

> "We have often heard it maintained that sciences should be built up on clear and sharply defined basic concepts. In actual fact no science, not even the most exact, begins with such definitions. The true beginning of scientific activity consists rather in describing phenomena and then in proceeding to group, classify and correlate them. Even at the stage of description it is not possible to avoid applying certain abstract ideas to the material in hand, ideas derived from somewhere or other but certainly not from the new observations alone. Such ideas—which will later become the basic concepts of the science—are still more indispensable as the material is further worked over (Freud, 1915)" (Instincts and their Vicissitudes. 1915).

Sigmund Freud established psychoanalysis, which continued to develop and spread worldwide within and outside psychiatry until the 1970s. At the same time, neuroscience and biological psychiatry have followed their own developmental paths. Psychopharmacological treatments had become widely accepted for mental illness since the 1970s and by the 1980s, psychoanalysis was regarded to be outdated, even unscientific (Wolpert and Fonagy, 2009; Fonagy and Lemma, 2012; Salkovskis and Wolpert, 2012). However with the rethinking of Freudian concepts, neuroscience has recently started to refocus upon psychoanalytic theories in the novel field of neuropsychoanalysis (Fonagy, 2001; Solms and Lechevalier, 2002; Solms and Turnbull, 2002; Panksepp, 2007; Arminjon et al., 2010; Northoff, 2011; Panksepp and Solms, 2012).

As a matter of fact, Freud himself began his career as a neurologist, and was a leading neuroscientist in the late 19th century before his establishment of psychoanalysis. His neuronal network idea at that time can actually be found in his private letters to Wilhelm Fliess. Charles Scott Sherrington, a famous British physiologist, discovered gaps between neurons and called them "synapses" in 1887. Two years before the Sherrington's discovery, Freud sketched synapse-like drawings and the existence of a energy source in his private letter (**Figure 1**) (Freud, 1950 [1895]). This fact highlights Freud's foresight in neuroscience. However, after he had established psychoanalysis, he devoted himself to developing not neuroscientific but psychological theories, and never published his schemes of neurons during his life (Northoff, 2012). Could it be possible that Freud might have dreamed of biological explanations of the mind that would one day replace psychological ones? If he had lived in our modern era, he might have proposed such a hypothesis to modern neuroscientists.

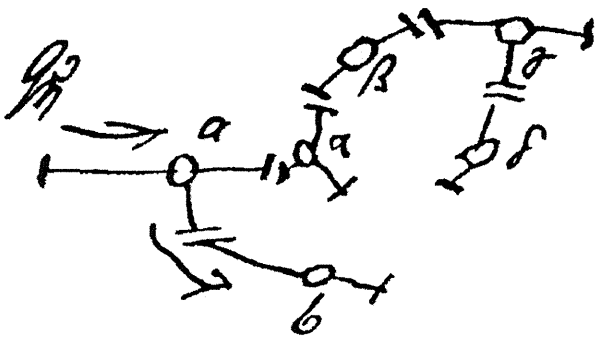

FIGURE 1 | Freud's sketch of the neuronal mechanism of the mind. Freud proposed a neuronal mechanism of repression in 1895. This is the famous sketch of his idea. He forecasted the existence of the "synapse" in this sketch. According to his scheme of unpleasant memory, a stimulus (Qη) normally activates unpleasant memory from neuron "a" to neuron "b"; however if other neurons ("α" and "β") exert a "repressing" influence, such activation is prevented. Based on our microglia theory, the function of "Qη" and/or "α" could be equivalent to a function of microglia as an energy source. Not only "repression" but also other unconscious functions, which were discovered by Freud and later psychoanalysts, may be modulated by microglia.

IS THE MIND A COMPUTER? DO COMPUTERS NEED ENERGETIC DRIVES?

Various hypotheses have been investigated to clarify the relationship between the mind and the brain; however the underlying mechanism remains unresolved. Traditionally, from a neuroscience perspective, the mind has been regarded to consist of neurons and neuronal circuit systems including synapses in the brain in the same way that computers consist of intricate metallic circuitry, and this view has persisted to the present day. In addition, the pathophysiology of mental illness has also been regarded to be within the context of neuronal circuit disturbances via neurotransmitters. On the other hand, a century ago, Freud proposed an energy model of the unconscious mind initially described as "Qη" (**Figure 1**) (Freud, 1950 [1895]), although this idea has been greatly ignored within the neuroscientific world. However, the computer itself does not work without energy and will only work adequately when the energy systems, such as heating/cooling, operate appropriately. Similar to a computer system model, additional energy systems in the brain may be needed to operate the mind. Originally, Freud and psychoanalytic researchers conceptualized "energy" similar to the thermodynamic conception of energy that all energy tends to ultimate equalization and stabilization and that, therefore, units of higher energy content within a system of lower energy content are unstable and tend to degrade, by importing physics theories from metapsychological perspectives (Freud, 1895, 1933a; Bernfeld and Feitelberg, 1931; Penrose, 1931; Erdelyi, 1985). On the other hand, recent biological studies have suggested that inflammation and oxidative stress, two of the most important energies in the brain, play important roles in the pathophysiology and interventions of various mental illnesses (Ng et al., 2008; Kato et al., 2011a; Maes et al., 2011, 2012). Herein, we propose a novel theory of an unconscious mind structural system in the brain by importing the role of microglia as the energy source and modulator of the brain from a neuropsychoanalytic approach (Solms and Lechevalier, 2002; Solms and Turnbull, 2002; Panksepp, 2007; Northoff, 2011; Panksepp and Solms, 2012).

WHAT ARE MICROGLIA?

Microglia, which were initially discovered by del Rio Hortega in 1919, are one of the glial cells in the brain. Traditionally, neuroscientists regarded microglial function as simply providing physical support and maintenance for neurons. Thus, in this limited role microglia had been long ignored (Miller, 2005). The last 20 years have elucidated various biological functions of microglia that act as "brain macrophage"; crucial immunological/inflammatory players in the brain by moving around and releasing cytokines and free radicals (Block et al., 2007; Hanisch and Kettenmann, 2007). Thus, microglia have proved to play more important roles in normal brain functions and various brain pathologies such as neurodegenerative diseases and neuropathic pain via inducing inflammation and oxidative stress (Inoue and Tsuda, 2009; Graeber, 2010; Graeber and Streit, 2010; Kettenmann et al., 2011; Ransohoff and Stevens, 2011).

PSYCHIATRIC DISORDERS AND MICROGLIA

Inflammation, oxidative stress, and immunological abnormality have been highlighted in various psychiatric disorders (Ng et al., 2008; Pasco et al., 2010; Kato et al., 2011a; Maes et al., 2011, 2012; Davison, 2012; Nicholson et al., 2012). The pathophysiology of psychiatric disorders has been dominantly believed to be solely explained by abnormalities of neurotransmitter systems. While, recent brain imaging and histological studies have indicated microglial activation in the brain of people with psychiatric disorders such as schizophrenia, depression, and autism (Radewicz et al., 2000; Steiner et al., 2008; Van Berckel et al., 2008; Doorduin et al., 2009; Morgan et al., 2010; Takano et al., 2010). Psychotropic drugs have long been regarded to have effects solely on neurons and neuronal networks including synapses, while our rodent *in vitro* studies have proved the novel effect of psychotropic drugs directly on microglia by suppressing release of inflammatory cytokines and free radicals (Kato et al., 2007, 2008, 2011a,b; Horikawa et al., 2010). Based on the abovementioned findings, we have proposed a microglial contribution to psychiatric disorders (Monji et al., 2009; Kato et al., 2011a). Immunological/inflammatory activators such as lipopolysaccharide (LPS) and interferon-γ, which are induced by infections, and various stressful life events, may activate microglia in the brain. Activated microglia release proinflammatory cytokines and free radicals (Block and Hong, 2005). In the brain of patients with psychiatric disorders, these mediators may cause brain pathologies such as neuronal degeneration, white matter abnormalities, and decreased neurogenesis (Uranova et al., 2004, 2007; Jarskog et al., 2005; Lieberman et al., 2005; Girgis et al., 2006; Glantz et al., 2006; Macritchie et al., 2010). Such remodelings of neuron-microglia interactions may thus be important factors in the pathophysiology of psychiatric disorders (Monji et al., 2009, 2011; Kato et al., 2011a).

STRESS, SUICIDE, AND MICROGLIA

Furthermore, recent animal studies indicate that microglia are activated not only under inflammation but also under physical stress (Frank et al., 2007; Sugama et al., 2007, 2009) and under psychosocial stress such as social isolation (Schiavone et al., 2009), chronic restraint stress (Tynan et al., 2010; Hinwood et al., 2012a,b) and social defeated situations (Wohleb et al., 2011). These data suggest that microglia may contribute not only to physical disturbance but also to emotional disturbance. Human postmortem studies have revealed microglial activation in the brain of suicide victims (Steiner et al., 2006, 2008). Suicide has generally been regarded as a byproduct of emotional disturbance, and furthermore, in the field of psychology and psychoanalysis, suicide has been considered to be the result of maladaptive unconscious drives. Herein, the question arises: Could microglia drive our unconscious drives? Before presenting a bridging theory between microglia and unconscious drives, we introduce the historical concept of these psychoanalytic drives.

THE CONCEPT OF PSYCHOANALYTIC UNCONSCIOUS DRIVES

A century ago, Freud proposed the conception of mind structure models consisting of the following three components: *the id* (unconscious/instinctual drives), *the ego* (the exclusive apparatus of the conscious mind), and *the super ego* (which represses *the id* in order to avoid any disruptions of rational thought). In the process of clarifying the unconscious components—*the id* and *the super ego*, Freud additionally developed the economic energy models of the following unconscious drives; first the "*life instinct (Lebenstrieb)*"—the tendency toward survival, propagation, and other creative life-producing drives, and later the "*death drive (Todestrieb)*" described in "*Beyond the Pleasure Principle* (Freud, 1920)" as "*... everything living dies for internal reasons—becomes inorganic once again—then we shall be compelled to say that 'the aim of all life is death' and, looking backwards, that 'inanimate things existed before living ones'*".

Following Freud's discovery of *the death drive*, it has continued to be one of the key concepts of psychoanalysis, which is often considered to form the basis of various emotions/behaviors—anxiety, fear, aggression and envy, and problematic behaviors including violence and suicide (Freud, 1933b; Klein, 1957). Historically, Freud underpinned *the death drive* from clinical phenomena such as negative therapeutic reactions, repetition-compulsion, anxiety dreams in persons with war neurosis, and masochism. Freud considered that *the life instinct* and *the death drive* fuse together in early life stages, and emphasized that *the death drive* was silently driving individuals toward death and that only through the activity of *the life instinct* was this death-like force projected outwards and appeared as destructive impulses directed against objects in the outside world (Freud, 1924). Freud named the outward-directed death drive "the destructive instinct (drive)." Melanie Klein and Karl Menninger were among the very few psychoanalysts who succeeded and developed the concept of *the death drive*. Klein, the Vienna-born British female psychoanalyst, who further developed Freud's concept of *the death drive* and was the basis of the Kleinian school in her later life, regarded the super ego in early life stages as the clinical expression of *the death drive* (Klein, 1932). Based on her theory, humans genetically and potentially have both *the life instinct* (desires for affection and/or objects) and *the death drive* (destructiveness and aggression), and these drives are expressed as internal/external object relations (good object/bad object) (Klein, 1957). Klein and Hanna Segal, a prominent Kleinian psychoanalyst, linked *the death drive* to envy (Segal, 1952, 1993). Segal also linked it to aesthetics by describing that "*Re-stated in terms of instincts, ugliness—destruction—is the expression of the death instinct; beauty—the desire to unite into rhythms and wholes, is that of the life instinct. The achievement of the artist is in giving the fullest expression to the conflict and the union between those two* (Segal, 1952)." Herbert Rosenfeld regarded the death drive in line with the concept of the *pathological organization* (*narcissistic organization*) in which good objects are abolished and destroyed internally in the self (Rosenfeld, 1971). As stated above, Kleinian theory has been continuously developed based on two opposing internal objects; the good object and the bad object. On the other hand, independent group psychoanalysts have developed their own theories. Ronald Fairbairn avoided the good/bad dichotomy, and established a unique object-relation theory with two essential objects; the exciting object and the rejecting object (Fairbairn, 1952). He assumed that the two internal objects were the roots of human behaviors and emotional life. Donald Winnicott emphasized the importance of external objects (environmental factors) in addition to internal objects (Winnicott, 1953, 1960).

Researchers such as Heinz Hartmann, Otto Kernberg, and Jaak Panksepp have fundamentally discussed the concept of instincts and drives in psychoanalysis in connection with biology and affective neuroscience. Hartmann, one of the founders of ego psychology, developed the theory of aggression based on *the death drive* (Hartmann, 1939). In addition, Panksepp, who coined the term "affective neuroscience," has been proposing a provocative theory linking drives and emotions. Based on his neurobiological and neuropsychoanalytic background, he and his colleagues have recently developed the theory of the SEEKING system (Wright and Panksepp, 2012). The SEEKING system is described as a "primary process" that promotes psychomotor eagerness to obtain pleasure generating resources and eliminate calamities, providing euphoric anticipatory excitement, and linking with other drives, such as those apart of the rewarding affective systems of LUST, CARING, and PLAY, and at times the aversive affective systems of FEAR and RAGE (Wright and Panksepp, 2012). Interestingly, in the commentary of the article of Wright and Panksepp, and Kernberg suggested "the concept of 'death drive' be retained for the pathological predominance in some clinical conditions of negative internalized object relations that may lead to an overwhelming dominance of self-destructive motivation (Kernberg, 2012)."

In psychoanalysis, the relationship between Es (id), libido and drive (instinct) has been ambiguously classified. While valuing Freud's original concept of the two essential drives and the following psychoanalytic theories, we believe that these concepts should be modified with accordance to recent theoretical/biological developments as discussed above. In the present day, the majority of psychoanalysts and scholars such as ethologists and

experimental psychologists are skeptical regarding the validity of *the death drive* as a relevant concept (Dufresne, 2000), but many researchers continue to accept the concept of the (aggressive) destructive drive (Rosenfeld, 1971; Feldman, 2000; Britton, 2003; Kernberg, 2012). In this article, we use the term of *the death drive* basically as the destructive drive (instinct), which induces negative emotions and outward destructive behaviors. In the following part, we propose a novel integrating theory of unconscious drives in order to fit both psychoanalytical and biological models.

BRIDGING THEORY BETWEEN MICROGLIA AND UNCONSCIOUS DRIVES—DO MICROGLIA DRIVE HUMAN MENTAL ACTIVITIES AS THE ORIGIN OF UNCONSCIOUS DRIVES?

To our knowledge, the internal reasons of the death drive have never been clarified from a molecular neuroscientific perspective. We herein propose a novel challenge to dig up the underlying mechanism of the drives with the modern understandings of microglia and their immunological roles in the brain. Obviously, Freud would not have known of such cells, however surprisingly, he implied a linkage between immunity and suicide in the following sentence:

> "...It is noteworthy that the obsessional neurotic, in contrast to the melancholic, never in fact takes the step of self-destruction; it is as though he were *immune* against the danger of *suicide*, and he is far better protected from it than the hysteric (Freud, 1920)."

In the present day, the role of microglia has been understood with a greater clarity than in Freud's era. Synaptic reactions have for a long time been regarded to play an essential role in human mental activities, while only neurons have been highlighted. Now, rodent microglia have proved to contribute to brain development such as synaptic pruning (Paolicelli et al., 2011), which suggest that microglia may play an important role in the process of brain development. Other animal studies have shown that microglia monitor synaptic reactions via direct-touching even in the normal brain (Wake et al., 2009; Graeber, 2010; Ransohoff and Stevens, 2011). Interestingly, some synapses in the ischemic areas disappear after a prolonged microglial contact (Wake et al., 2009), which may suggest that severe mental stress induces synaptic changes via microglial responses. Recent rodent studies have reported that severe stresses including psychosocial stress activate microglia (Frank et al., 2007; Schiavone et al., 2009; Sugama et al., 2009; Tynan et al., 2010; Wohleb et al., 2011; Hinwood et al., 2012a,b). In addition, human studies suggest that microglial activation is observed in the brain of psychiatric patients and suicide victims (Steiner et al., 2006, 2008; Van Berckel et al., 2008; Doorduin et al., 2009; Takano et al., 2010). Under these microglia-activated states, unconscious drives could be highly activated from a psychoanalytic perspective.

In sum, a novel hypothetical theory arises: "When microglia is maladaptively activated in the brain, microglia may act as the origin of unconscious drives such as *the death drive* in the unconscious mind, and induce emotional reactions such as anxiety, fear, aggression, envy, and suicidal thought/behaviors (**Figure 2**)."

TRANSFERENCE, PSYCHOLOGICAL IMMUNE MEMORY/REACTION, AND MICROGLIA

One of the essential lessons of psychoanalysis represented by the Oedipus complex is that psychological experiences during childhood between people closely related (i.e., mother, father and siblings) last until adulthood, (Freud, 1900, 1905). Unconscious reactions, which are memorized during childhood, are reflected onto immediate persons in various settings such as home, school, and work. These unconscious reactions occurring toward psychoanalysts are called transference; e.g., a client felt enraged toward his psychoanalyst, as he would have experienced toward his father in childhood. Dealing with transference is a major therapeutic approach of psychoanalysis. Psychoanalysts would interpret that his unconscious aggressive drive produced by the father–child relationship is reproduced during the here and now psychoanalyst-client relationship. Owing to such an approach, the client may recognize his own unconsciously derived aggression and he may be released from it.

Transference and its underlying mechanisms can be explained within the paradigm of microglial priming. Bilbo and Schwarz suggest that microglial activation due to infections during early developmental periods last, and these pre-activated microglia will be re-activated rapidly compared to normal state microglia as microglial immune memory (Bilbo and Schwarz, 2009). Interestingly, Bilbo and her colleagues recently reported that early life stress in the rat influence formation of memories in later life by microglial immune memory (Williamson et al., 2011).

Various stressors, not only infection but also psychosocial stress, may be memorized inside the microglia during childhood as the origin of unconscious drives, which we have dubbed "psychological immune memory." In later life, various similar stressors re-activate the microglia and lead to transference-like situations; emotional reactions during childhood (i.e., traumatic events) are reproduced afterwards as "psychological immune reactions" (**Figures 3** and **4**). The underlying mechanism of Post-Traumatic Stress Disorder (PTSD) could also be explained by this process. Interestingly, Klein proposed the "memories in feelings" in her representative book "Envy and Gratitude (Klein, 1957)." The word "memories in feelings" means that strong primitive feelings themselves during childhood are memorized psychologically, and these feelings are reenacted in later life as transference. Klein described such feelings as follows:

> "All this is felt by the infant in much more primitive ways than language can express. When these pre-verbal emotions and phantasies are revived in the transference situation, they appear as 'memories in feelings', as I would call them, and are reconstructed and put into words with the help of the analyst. In the same way, words have to be used when we are reconstructing and describing other phenomena belonging to the early stages of development. In fact we cannot translate the language of the unconscious into consciousness without lending it words from our conscious realm (Klein, 1957)."

This Kleinian mechanism may also be explained by our microglia theory of the psychological immune memory/reaction. Recent epidemiological studies have revealed that maladaptive

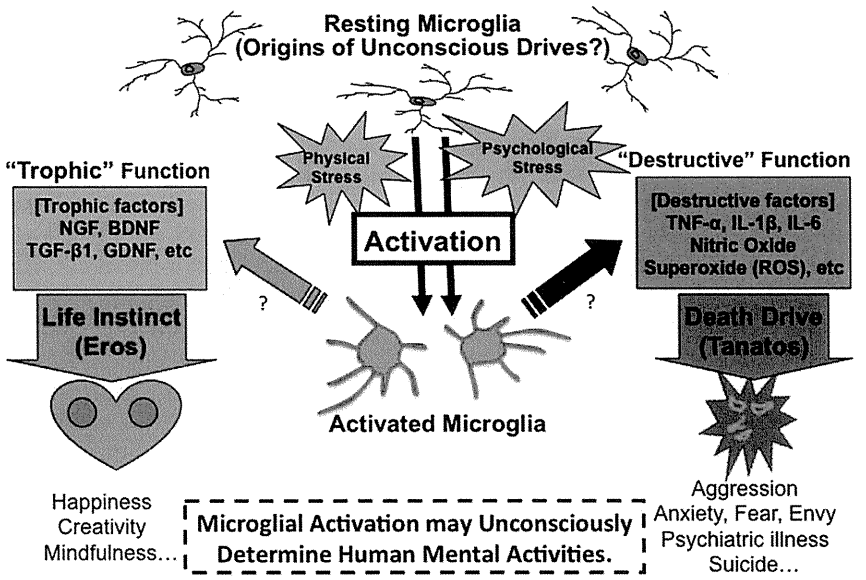

FIGURE 2 | Microglia theory of unconscious drives. Microglia may act as the origin of unconscious drives in the mind. Microglia play the role of a double-edged sword in the brain. Microglia release not only maladaptive factors such as TNF-α but also protective factors such as BDNF, which means that microglia are alternately both bad and good actors in the brain. The direction and strength of microglial activation may be the origin of Freud's two essential drives of death (tanatos) and life (eros). "Destructive" function of microglia may play an essential role in *the death drive*. Maladaptive microglial activation caused by a certain psychological/physical stress may induce aggressive, anxious, fearful, and envious states: these states may furthermore lead to psychiatric disorders and suicidal thought/behaviors. On the other hand, "Trophic" function of microglia may play an important role in *the life instinct*. Appropriate stress may activate microglia at good enough levels, which induce protective factors and result in happy, creative and mindful states.

parent-child relationships and childhood trauma are the crucial risk factors for psychiatric disorders in later life (Alvarez et al., 2011; Bebbington et al., 2011; Hovens et al., 2012; Morgan et al., 2012). In addition, a recent report of a human twin study suggests that childhood trauma induces inflammatory reactions (Rooks et al., 2012). Such evidence supports our proposed theory that microglial immune memory may develop psychiatric disorders in later life.

The origin of unconscious processes in the brain especially in psychiatric condition has not been well understood. Our theory may reflect a heightened attempt by microglia to achieve homeostasis in the brain when it is under physical or psychosocial stress. In the process of understanding emotional systems in the brain, neuronal centered explanations have been dominant including the importance of schemata, higher-order conditioning, implicit memory, and experience-dependent shaping of neurotransmitter systems (Solms and Turnbull, 2002; Panksepp, 2004; Welzer and Markowitsch, 2005; Wright and Panksepp, 2012). At present, the connection between the immunological role of microglia and our proposed "psychological immune memory" has not been well clarified. However, a series of studies by Bilbo and her colleagues (Bilbo and Schwarz, 2009; Williamson et al., 2011) and other recent thought-provoking animal studies have suggested interesting physiological outcomes regarding microglial contribution to psychological immune memory and emotional responses. As shown the above, rodent studies have reported that severe stresses including psychosocial stress activate microglia (Frank et al., 2007; Schiavone et al., 2009; Sugama et al., 2009; Tynan et al., 2010; Wohleb et al., 2011; Hinwood et al., 2012a,b). Acute stress is demonstrated to induce morphological microglial activation in several brain regions including the midbrain periaqueductal gray (PAG), an area that plays crucial roles in behavioral and emotional responses to uncontrollable stress, threat, anxiety, and pain. Sugama et al. determined whether neuronal activation may be involved in the stress-induced microglial activation by measuring the correlation between neuronal activity measured as c-Fos expression and morphological microglial activation in the PAG (Sugama et al., 2009). Acute stress was succeeded by morphological activation of microglia and increased c-Fos expression in the PAG, and their analysis demonstrated that microglial activation occurred adjacent to responsive neurons. By contrast, LPS treatment induced microglial activation even in the absence of neuronal responses in the PAG as well as in the rest of the midbrain. Their findings suggest that the mechanism of microglial activation during stress may differ from those of infection or inflammation. Based on their results, Sugama et al. suggested that stress-induced c-Fos protein from activated neuronal cells may play some roles to trigger microglial activation. Recently, Hinwood et al. investigated a series of rodent studies how psychological stress affects microglia (Hinwood et al., 2012a,b). They found that chronic psychological stress increases the internal complexity of microglia, and that chronic stress markedly increases the expression of beta1-integrin (CD29), a protein previously implicated in microglial

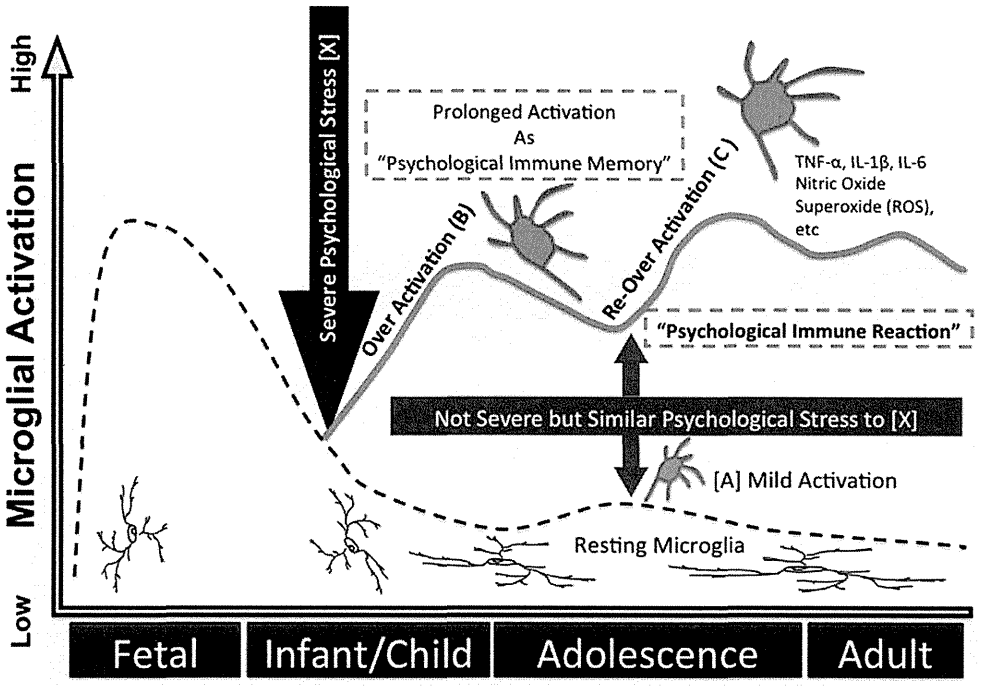

FIGURE 3 | Developing process of "psychological immune memory/reaction" via microglia. Microglia have proven to have direct connections with neuronal synapses, and external stress activates microglia via neuronal stimulus. Not only physical stress but also psychological stress activates microglia in animal models. Herein we propose a possible process of microglia activation as "psychological immune memory" during human life. **(A)** Normal daily psychological stress (not extreme stress) moderately and temporarily activates microglia via neuronal stimulus **(A)**. **(B)** Severe psychological stress (e.g., trauma) over-activates microglia via nervous excitement **(B)**, which induces a storm of inflammatory cytokines and free radicals in the brain, thus resulting in abnormal behaviors and strong emotional reactions. In addition, this storm results in damaging neuron-glial networks and the subsequent rebuilding of novel neuro-glial networks. This network change means that previous psychological reactions dramatically change into novel stimulus-output patterns. Moreover, once microglia have been activated strongly, these microglia remain in pre-activated states for years, which we can dub "**psychological immune memory.**" **(C)** Pre-activated microglia **(B)** are excessively responsive to even slight stimuli when the stimuli are similar to the previous traumatic stress, and are again over-activated **(C)**. Similar but slight stress, which was previously only a good enough modulator of microglia, results in a strong storm of the brain/abnormal behavior/strong emotional reaction as that of the previous traumatic reaction. We have dubbed this reaction "**psychological immune response,**" and it can explain many psychological and psychopathological mechanisms such as transference and repeated behavioral/emotional reactions typically seen in PTSD.

ramification (Hinwood et al., 2012b). These findings suggest that beta1-integrin may be one possible modulator between psychological stress, neuronal network activity and microglial ramification (Hinwood et al., 2012b). Above-mentioned animal studies indicate that unconscious drives may involve both activated neurons and/or activated microglia, while it is very difficult to differentiate between clusters of neuronal activation and microglial activation in the process of unconscious brain processes and emotional motivations because of the difficulty of establishing experimental models. Furthermore, to our knowledge, it is also difficult under current scientific conditions to clarify whether microglia are the underlying precipitator of unconscious thought processes and motivations. To our knowledge, the exact process of how microglial and/or neuronal activation affect emotional experience and behavior has not been well understood. Interestingly, a recent animal study has suggested that microglial activation has a positive link to anxiety-like behaviors, and suppressing microglial activation by minocycline results in ameliorating the anxiety-like behaviors (Neigh et al., 2009). This report suggests that microglial activation may, at least to some extent, contribute to the occurrence of anxiety. As introduced the above, microglia are recently known to have continuous direct contact with synapses (Wake et al., 2009). In addition, microglia are known to have various neurotransmitter receptors, and neurotransmitters are reported to affect not only the neuronal system but also microglia (Pocock and Kettenmann, 2007; Kato et al., 2013). Therefore, in our opinion, microglial activation may induce a disturbance of neuron-microglia communication at least to some extent, and neuronal systems, which organize emotional and psychological experience and behavior, may be over-activated. Further studies should be conducted to clarify how microglial activation affects neuronal system, emotional and psychological experience and behavior.

Microglial psychoimmunological memory is a novel concept which we have just recently proposed. To our knowledge, no study has been conducted in this aspect. Traumatic memories may be located within neural networks without having to recur to microglia, or microglia may contribute much to such

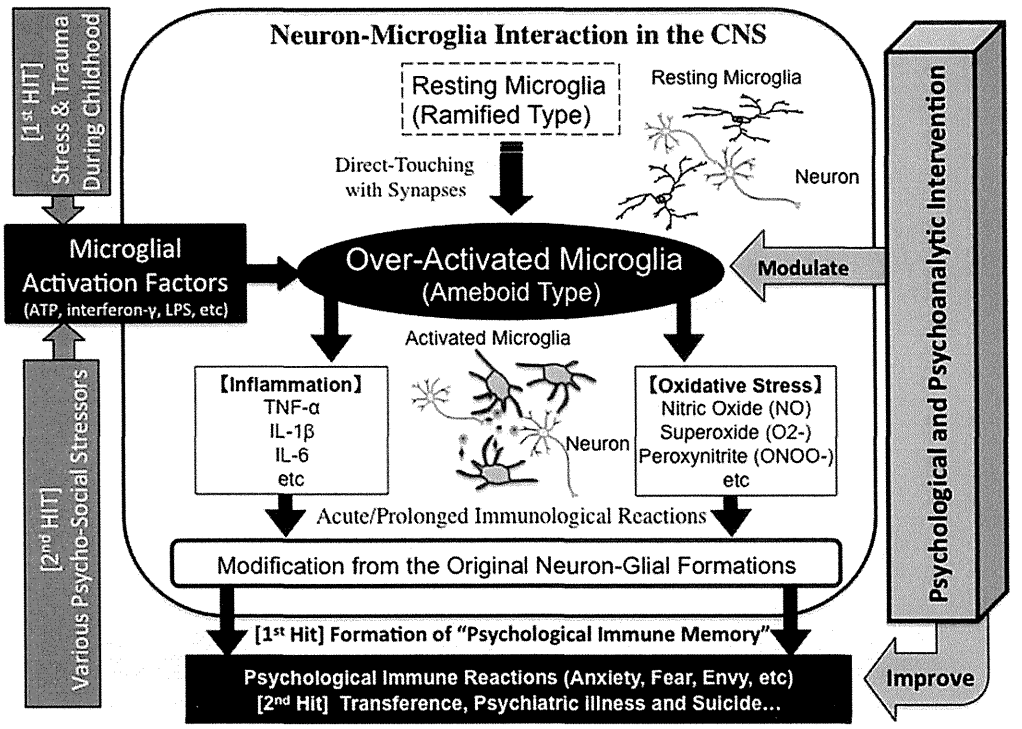

FIGURE 4 | Neuron-microglia interaction during "psychological immune memory/reaction." Strong psychosocial stress such as trauma during childhood may over-activate microglia, which induce a variety of inflammatory/oxidative-stress factors and result in damaging the original neuron-microglial formations. Finally, novel neuron-microglial networks will be formulated. This reaction is memorized as "psychological immune memory". When similar psychosocial stress, even at weaker levels, occurs in later life, the primed-microglia may be over-activated. This reaction will also induce various maladaptive psychological reactions, which may result in transference reactions during interpersonal relationships, psychiatric disorders and also suicidal behaviors. On the other hand, psychological and psychoanalytic interventions may improve these states by suppressing microglial maladaptive activation.

memories. Further studies are needed to clarify the relationship between early trauma, emotional behavior and microglial immune reactions.

SOCIAL INTERACTION AND MICROGLIA IN HEALTHY HUMAN

Until recently no experiment had been conducted focusing on human social and psychological factors in relation to microglia, and there is no known drug with the specific effect of modulating human microglia. Therefore, using minocycline, a tetracycline antibiotic and the most famous microglial inhibitor in rodent models, is one of the best alternative approaches to clarify microglial functions in human social/mental activities. A recent rat study has shown that minocycline suppresses microglial activities not only in stress-induced activation states but also in resting states (Hinwood et al., 2012b). In order to examine how microglia influences social and mental activities, we recently examined how minocycline works in human social decision-making by trust game (Watabe et al., 2012); healthy adult males made a monetary decision about whether or not to trust an anonymous partner after a 4-day oral administration of minocycline. The minocycline group showed a positive correlation between their monetary score in the trust game and their evaluation scores of others' trustworthiness in a questionnaire, but surprisingly the placebo group did not. Thus, minocycline sharpened participants' sense of trust that led them to be more decisive in the game. This first trial has suggested that microglial activation may cause "unconscious noises" against appropriate social decision-making, and inhibiting microglial activity may reduce such noise (Watabe et al., 2012). In a subsequent trial with larger samples, we additionally measured the effects of anxiety and personality (Kato et al., 2012). The monetary score in the trust game was significantly lower in the minocycline group. Interestingly, participants' ways of decision-making were significantly shifted; cooperativeness, one component of personality, proved to be the main modulating factor of decision-making in the placebo group, on the other hand, the minocycline group was mainly modulated by state anxiety and trustworthiness. Our results of the second trial suggest that minocycline led to more situation-oriented decision-making, possibly by suppressing the effects of personality traits, and furthermore that personality and social behaviors might be modulated by microglia. Early life events may activate human microglia, establish a certain neuro-synaptic connection, and this formation may determine personality and personality-oriented social behaviors in later life (Kato et al., 2012).

The above-mentioned findings shed new light on the dark side of microglial social/mental functions in humans, especially highlighting the role of microglia for the unconscious. In the same way that Freud proposed that our behaviors must be controlled by the unconscious world, microglia may unconsciously control our behaviors. Human neuroscience focusing not only on computer-like neuronal networks but also on "noisy" microglia would be a novel key for investigating "noisy" human social/mental activities that are unlike "noiseless" computers. To explore these mechanisms, further translational research is needed.

MICROGLIAL DOUBLE-EDGED SWORD AND AMBIVALENCE

Microglia play an interesting role as a double-edged sword in the brain (Henkel et al., 2009; Graeber and Streit, 2010). Microglia release not only maladaptive factors such as Tumor Necrosis Factor (TNF)-α but also protective factors such as Brain-Derived Neurotrophic Factor (BDNF), which means that microglia are alternately both bad and good actors in the brain. "Destructive" function of microglia may play a vital role in *the death drive.* On the other hand, "trophic" function of microglia may play an equally essential role in *the life instinct.* It remains controversial as to whether the origin of the two drives is the same from the psychoanalytic perspective. Based on our microglial theory, the origin and the determinant factor may be the composition and the direction of the microglia. Microglia are known to express different *faces* during developmental, adolescent and adult stages. The balance-shift of the trophic/destructive expression of microglia may explain the underlying origin of the two drives in the mind. The existence of two directional microglia in the same region may induce an ambivalence, which means a dilemma between the two directional emotions such as "love and hate." The direction of microglial activation may determine our behaviors toward life or death (**Figure 2**).

Our terms "trophic" and "destructive" microglia should not be taken in a strictly literal sense. Our proposed theory may be too oversimplified in implying that the function of microglia easily divides into (A) "trophic" function of microglia = preserving = the life instinct, and (B) "destructive" function of microglia = destroying = the death instinct. This dichotomy is not always true in real situations. Some microglia might destroy for synaptic pruning, which in the long run is a trophic result for the brain, to preserve energy for more frequently functioning neuron populations, and to reconstruct more appropriate neuronal networks. Furthermore, we could apply this proposed neuroscientific process into human psychological development as follows: It is somewhat essential to have painful/stressful experiences in developing periods, during which microglia may activated, and neuronal networks may be reformed, and finally rebuilt a more prosocial personality and/or resilient self in later life. However, for some, this process might not work through, and result in pathological/psychiatric conditions. It is not known how differentiate destructive processes that are useful, from destructive processes that are associated with pathology, while we prospect that these different outcomes might be determined by factors such as genetic vulnerability, extremely painful/stressful events, dysfunction of neurons/microglia, and environmental factors before/after these events. For example, some volume of microglia-releasing mediators such as pro-inflammatory cytokines and/or free radicals may be essential for our mental development; however microglia in some individuals may easily release too much of such mediators even after weak stressful events. Those individuals may easily be prone to psychiatric conditions. At least to some extent, recent neuropsychoanalytic theories such as the Panksepp's SEEKING system (Wright and Panksepp, 2012) and Kernberg's "death drive" theory (Kernberg, 2012) may be complemented by our proposed microglial theory. Digging up these interactions provide for further translational research opportunities to bridge the huge gap between the brain and the mind. Aging is known to be one of the key switching factors of microglial characteristics. Generally speaking, aging tends to activate microglia maladaptively (Dilger and Johnson, 2008; Jang and Johnson, 2010; Norden and Godbout, 2012), which may provide a clue to clarify these underlying mechanisms.

POSSIBLE MICROGLIAL CONTRIBUTION TO THE CONSCIOUS AND THE UNCONSCIOUS WORLD

It is of great importance to understand the present situation of affective neuroscience and neuropsychoanalysis including the biological understanding of the unconscious/conscious. To our knowledge, all previous research has been focused solely on neuronal systems including synapses to understand the emotional reactions and the unconscious in the brain. It is a novel challenge to consider the role of microglia in emotional reactions and the unconscious. Neuronal systems and neurotransmitters have been regarded to have important roles in "unconsciously" modulating emotions and motivational behaviors (Solms and Turnbull, 2002). In addition, microglia may be one possible source of "unconsciously" generated negative emotions that do not directly rely on perceptual input but are generated biologically. Herein we hypothesize a possible role for microglia in emotional reactions. The following three processes might be occurring at least in some biological pathways of the unconscious/conscious; (Process I) microglia may be activated by neurotransmitter modulations connected with emotional reactions based on perceptual inputs, (Process II) microglial activation may modulate synaptic reactions via neurotransmitters resulting in emotional reactions, and (Process III) a mixed process of I and II may occur especially during continuous high emotional responses, in which primary emotional reactions may activate neuronal systems via synapses and neurotransmitter modulations, resulting in microglial activation, and finally mutual activation may occur via neurotransmitters and microglial mediators such as free radicals and/or cytokines. We hypothesize that process III may be one of the possible causes for emotional disturbance, symptoms of various psychiatric disorders and also suicide.

In addition, we now present a possible mechanism of the conscious and the unconscious in the brain. The system of the relationship between the conscious and the unconscious has long been considered within the context of neuronal systems. Microglia are now known to be very unique dynamic cells in the brain, which can move around and are usually independent from neuronal systems, and sometimes have direct contact with

synapses. These roles seem to be similar to Freud's perceptual theory called "the system Pcpt.-Cs., or the system W-Bw, which was named after the German words Wahrnehmung (= perception; Pcpt.) and Bewußtsein (= consciousness; Cs.)" (Freud, 1920). We suppose that microglial activation itself does not directly equate to emotional reactions, but we suggest that microglial activation may be one of the crucial priming factors of the unconscious for emotional reactions by affecting neuronal systems. It is easily understood that external inputs trigger emotional reactions, while the mechanism of emotional reactions without external input such as nightmares has not been fully comprehended. Our theory may shed new light on the understanding of internally caused (or the unconscious-derived) emotional reactions. Interestingly, microglial contribution has recently been suggested in the occurrence of delirium, which induces disturbance of the conscious by internal causes such as systemic infections (Van Gool et al., 2010). Our theory might give us the chance to re-translate Freudian theory of the system between the conscious and the unconscious. Further studies should be highlighted in this aspect.

CONCLUSION

FUTURE PERSPECTIVES

In this paper, we showed the possibility that microglial activation in the brain activates unconscious drives in the mind. We also presented the brain/mind structural system of ambivalence, transference, psychological trauma and even the Oedipus complex by importing the microglia theory of "psychological immune memory/reaction." In addition, we introduced a recent human study focusing on the microglial role of social decision-making. Finally, we showed a possibility that direction and context of microglial activation may be a key factors in our mental activities including unconscious world.

Previously, Eric Kandel explored the neuron-synaptic world based on his psychoanalytic background as a novel work of the 20th century (Kandel, 1979, 1999, 2005). In a similar mode to Kandel's exploration, the novel scientific field, now highlighted as "neuropsychoanalysis" (Fonagy, 2001; Solms and Lechevalier, 2002; Solms and Turnbull, 2002; Panksepp, 2007; Northoff, 2011; Panksepp and Solms, 2012), has endeavored to clarify the underlying mechanism of the unconscious and psychoanalytic theories from a neuroscientific perspective. In the 21st century, new challenges focusing on microglia should be explored in the new world of the mind/brain beyond Kandel's neuron-synaptic doctrine. We believe that our proposed theory sheds new light on solving deeper mechanisms of "unconscious drives" from both psychoanalytic and neuroscientific perspectives. Microglia may have the potential to bridge the huge gap between neuroscience, biological psychiatry, psychology, and psychoanalysis. Further communication between neuroscientists, psychiatry, psychologists, and psychoanalysts is required. To investigate the microglia theory, further translational research from *in vitro*/*in vivo* animal studies to human studies is needed based on the neuropsychoanalytic approach. Finally, we highlight some research questions of particular importance to be clarified:

- What is the key interaction between microglial activation (biological world) and the unconscious (psychological world)?
- What kind of afferent networks (afferent stimulus, input, impulse, etc.) and molecules such as neurotransmitters activate microglia under psychosocial stress?
- How do activated microglia act on efferent neuronal pathways, and how do microglia finally impact on the unconscious, emotions and behaviors? In relation to cognition, various studies suggest the positive link between microglial activation and dementia which is one of the most typical phenotypes of cognitive dysfunction, while the underlying mechanism between dementia's cognitive dysfunctions and microglial activation are less well understood. Can microglia modulate various cognitive functions under not only pathological states but also normal states? It is also unclear how microglia activation influences neurotransmitters and/or neural systems involved in emotional and experience and behavior and how microglial activation back-project to the mental and behavioral realm, while the following evidence may give a cue for future investigations. Not only neurons but also microglia have a variety of neurotransmitter receptors including dopamine and noradrenaline receptors (Pocock and Kettenmann, 2007; Kato et al., 2013), which are closely related to our mental activities and the pathophysiology of neuropsychiatric disorders. Sugama et al. showed that acute stress activates microglia in the PAG (Sugama et al., 2009). In addition, Neigh et al. suggested that microglial activation induce anxiety-like behaviors in mice (Neigh et al., 2009). These reports suggest that microglial activation may contribute to various emotional reactions.
- Microglia are thought to be a heterogeneous group. Therefore, we should investigate the actions of microglia in each group. Regional specificity might exist, and it may link to previously known understandings in psychiatric brain imaging studies.
- Microglial modification may create a novel strategy for intervention in psychiatric disorders. Clinical trials focusing on microglia should be conducted.
- Microglia have mutual communications not only between neurons but also astrocytes and oligodendrocytes. Therefore, mutual interaction of neuron-glia should be clarified to understand the deeper mechanisms of unconscious and neuropsychoanalytic theory in the brain.

FINAL REMARKS

Before developing psychoanalysis in the late 19th century, Freud sketched the neuronal mechanism of the mind (**Figure 1**), and Freud might have possibly dreamed that biological explanations of the unconscious mind would one day replace psychological ones (Freud, 1950 [1895]). Microglia may be a key player to realize Freud's long-unresolved dream.

ACKNOWLEDGMENTS

This work was initially presented at the ESF-JSPS Frontier Science Conference—"Social Cognitive Neuroscience" (March 2009, Acquafredda di Maratea, Italy), and was recently presented at the 11th/12th/13th International Neuropsychoanalysis Congress 2010/2011/2012 (Seattle, USA/Berlin, Germany/Athens, Greece). We thank Dr. Monji A. and Dr. Watabe M. for their valuable comments. This work was financially supported by the Japan Society for the Promotion of Science (JSPS).

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Received: 04 September 2012; accepted: 09 January 2013; published online: 04 February 2013.

Citation: Kato TA and Kanba S (2013) Are microglia minding us? Digging up the unconscious mind-brain relationship from a neuropsychoanalytic approach. Front. Hum. Neurosci. 7:13. doi: 10.3389/fnhum.2013.00013

frontiers in
INTEGRATIVE NEUROSCIENCE

HYPOTHESIS AND THEORY ARTICLE
published: 15 July 2013
doi: 10.3389/fnint.2013.00053

Missing and possible link between neuroendocrine factors, neuropsychiatric disorders, and microglia

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Endocrine systems have long been suggested to be one of the important factors in neuropsychiatric disorders, while the underlying mechanisms have not been well understood. Traditionally, neuropsychiatric disorders have been mainly considered the consequence of abnormal conditions in neural circuitry. Beyond the neuronal doctrine, microglia, one of the glial cells with inflammatory/immunological functions in the central nervous system (CNS), have recently been suggested to play important roles in neuropsychiatric disorders. However, the crosstalk between neuroendocrine factors, neuropsychiatric disorders, and microglia has been unsolved. Therefore, we herein introduce and discuss a missing and possible link between these three factors; especially highlighting the following hormones; (1) Hypothalamic-Pituitary-Adrenal (HPA) axis-related hormones such as corticotropin-releasing hormone (CRH) and glucocorticoids, (2) sex-related hormones such as estrogen and progesterone, and (3) oxytocin. A growing body of evidence has suggested that these hormones have a direct effect on microglia. We hypothesize that hormone-induced microglial activation and the following microglia-derived mediators may lead to maladaptive neuronal networks including synaptic dysfunctions, causing neuropsychiatric disorders. Future investigations to clarify the correlation between neuroendocrine factors and microglia may contribute to a novel understanding of the pathophysiology of neuropsychiatric disorders.

Keywords: microglia, endocrinology, corticotropin-releasing hormone, glucocorticoids, sex hormones, estradiol, oxytocin

INTRODUCTION

ENDOCRINE FACTORS AND NEUROPSYCHIATRIC DISORDERS

Endocrine systems play an important role in bridging the body and the brain via various hormones, and maintaining homeostasis in physical conditions, while hormone imbalances are known to induce various physical disorders (Selye, 1950; Chrousos and Gold, 1992). Endocrine organs, such as the adrenal gland and the gonads, communicate with the central nervous system (CNS) via neuroendocrine factors and contribute to a series of mental functions. The network between the hypothalamus, pituitary, and adrenal gland, called the Hypothalamic-Pituitary-Adrenal (HPA) axis, is one of the crucial pathways in stress response (Selye, 1950; Chrousos and Gold, 1992).

The HPA axis is related to a variety of psychiatric conditions, such as the depressive symptoms associate with Cushing syndrome and steroid-induced depression (Lynn, 1995; Terao et al., 1997), suggesting that the dysregulation of the HPA axis is possibly involved with the pathophysiology of depression (Roy et al., 1988; Lesch et al., 1990). On the other hand, reproductive functions especially during pregnancy, birth, and the postnatal period are exerted exclusively by the gonads through sex-related hormones. Sex hormones establish the estrous cycle, and affect mental activities. The disturbance of estrous cycle functions is closely involved with various symptoms of mood and emotion, frequently developing into mood disorders such as premenstrual dysphoric disorder (PMDD) (Steiner et al., 1995; Epperson et al., 2002) and depressive disorders during perinatal (Bloch et al., 2000) and perimenopausal periods (Freeman et al., 2004a). Interestingly, oxytocin, the neuroendocrine hormone mainly associated with the postnatal period, has recently been highlighted for its stress-suppression effects and prosocial functions. Oxytocin has been shown to be involved with anxiety and depressive mood in patients with depression (Scantamburlo et al., 2007). Oxytocin exerts anxiolytic effects through the reduction of stress-induced corticosterone release on rodents (Windle et al., 1997) and also on healthy humans exposed to stress (Heinrichs et al., 2003). Additionally, oxytocin has been suggested to be an important factor in mental development and its

Abbreviations: ACTH, adrenocorticotropic hormone; ADIOL, 5-androsten-3b,17b-diol; ASD, autism spectrum disorders; AVP, vasopressin; cADPR, cyclic-ADP ribose; CRH, corticotropin-releasing hormone; CRH-R1, CRH receptor I; CRH-R2, CRH receptor II; CtBP, C-terminal binding protein; ER, estrogen receptor; GABA, gamma-aminobutyric acid; HPA axis, hypothalamic-pituitary-adrenal axis; HPG, hypothalamic-pituitary-gonadal; IFN-γ, interferon gamma; IL, interleukin; LPS, lipopolysaccharide; MeCP2, *X*-linked methyl-CpG-binding protein 2; MS, multiple sclerosis; NO, nitric oxide; O&NS, oxidative and nitrosative stress; PMDD, premenstrual dysphoric disorder; PTSD, post-traumatic stress disorder; PVN, paraventricular nucleus; ROI, reactive oxygen intermediates; ROS, reactive oxygen species; TNF-α, tumor necrosis factor-α.

abnormality may be related to autism (Tetreault et al., 2012). The above-mentioned reports all suggest that the HPA axis-related hormones, sex hormones, and oxytocin have a possible link to psychiatric disorders, while the underlying biological mechanisms have not been well understood.

NOVEL UNDERSTANDINGS OF NEUROPSYCHIATRIC DISORDERS

Traditionally, abnormalities of neurons and neuronal networks including synaptic abnormalities and disturbance of neurotransmitters have dominantly been believed to be the main causes of psychiatric disorders. Beyond these classic understandings, novel theories have been presented that psychiatric disorders are systemic disorders widely driven by peripheral inflammatory and oxidative and nitrosative stress (O&NS) processes, and in addition that neuropsychiatric disorders are controlled by inflammatory, immune, O&NS, tryptophan catabolite, neuroinflammatory and neuroprogressive pathways in the CNS (Maes, 2011; Maes and Rief, 2012). Moreover, systemic inflammatory and O&NS processes heavily modulate hormonal levels, and reciprocal relationships between peripheral inflammation and hormonal levels are suggested to be involved in the pathophysiology of these disorders (Maes, 2011; Maes and Rief, 2012). In the brain, tryptophan catabolites such as kynurenine, kynurenic acid, 3-hydroxykynurenine, and quinolinic acid are synthesized in glia cells, and recent reports have shown that the blood kynurenine/tryptophan ratio is elevated in patients with depression and correlated with anxiety and cognitive disturbances (Schwarcz et al., 2012). Steiner et al. (2011) have shown pivotal findings that quinolinic acid positive microglia increase in the subgenual anterior cingulate cortex and anterior midcingulate cortex of patients with major depressive disorder (MDD) but not bipolar disorder. A relationship between the kynurenine pathway and depression is supposed to be induced by the activation of indoleamine 2,3-dioxygenase (IDO), an intracellular enzyme that catalyzes tryptophan degradation into kynurenine. However, how tryptophan catabolites correlate with depression pathology remains to be elucidated.

NEUROPSYCHIATRIC DISORDERS AND MICROGLIA

Macrophages in peripheral organs play important roles in the process of peripheral inflammation (Kiefer et al., 2001; Mosser and Edwards, 2008). Similarly, microglia, one of the immune cells in the brain, play crucial roles in neuroimmunology including neuroinflammatory and neuroprogressive pathways. Microglia were initially discovered by Del Rio-Hortega (1919). Traditionally, neuroscientists regarded microglial function simply to provide physical support and maintenance for neurons. Thus, microglia had been long ignored (Miller, 2005). However studies during the last 20 years have elucidated various biological functions of microglia that act as "brain macrophage"; crucial immunological/inflammatory players moving around, monitoring micro-environmental changes, and once activated, releasing pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and free radicals including nitric oxide (NO), and finally inducing neuropathologies such as phagocytosis, apoptosis of neuronal cells, suppressing neurogenesis, and oligodendrocyte dysfunction (Block et al., 2007; Hanisch and Kettenmann, 2007). Thus, microglia have proved to play important roles in neurodegenerative diseases and neuropathic pain (Inoue and Tsuda, 2009; Graeber, 2010; Graeber and Streit, 2010; Kettenmann et al., 2011; Ransohoff and Stevens, 2011).

Recent positron emission tomography (PET) imaging of peripheral benzodiazepine receptors and postmortem studies have suggested microglial activation in the brain of patients with neuropsychiatric disorders such as schizophrenia, depression, and autism (Steiner et al., 2006, 2008; van Berckel et al., 2008; Doorduin et al., 2009; Morgan et al., 2010; Takano et al., 2010; Tetreault et al., 2012; Suzuki et al., 2013). Interaction between microglial activation and psychopathology has not been well clarified, while a PET study has resulted in an interesting outcome; Takano et al. (2010) reported that chronic schizophrenia patients showed a positive correlation between cortical [11C]DAA1106 binding (one of the peripheral benzodiazepine receptors ligand) and positive symptom scores which were assessed using the Positive and Negative Syndrome Scale. Although the correlations need to be interpreted very cautiously, involvement of microglial activation in the pathophysiology of positive symptoms of schizophrenia might be suggested. Animal models of neuropsychiatric disorders such as schizophrenia and autism have proposed the underlying microglial pathologies (Juckel et al., 2011; Derecki et al., 2012; Liaury et al., 2012). In addition, various psychotropic drugs, which had classically been regarded to modulate solely neurons and synaptic networks, have recently been revealed to have direct anti-inflammatory properties on activated microglia in a series of *in vitro* studies (Hashioka et al., 2007; Kato et al., 2007, 2008, 2011b; Horikawa et al., 2010). Therefore, microglia may play crucial roles in the pathophysiology and treatment of neuropsychiatric disorders (Monji et al., 2009, 2013; Kato et al., 2011a, 2013).

In addition, minocycline, a tetracycline antibiotic, has recently been known to improve symptoms of psychiatric disorders such as schizophrenia (Miyaoka et al., 2007; Miyaoka, 2008; Levkovitz et al., 2010). Minocycline has a variety of functions in the CNS such as interacting with brain glutamate and dopamine neurotransmission (Kim and Suh, 2009) and having direct effects on neuronal cells (Hashimoto and Ishima, 2010). Rodent studies have revealed that minocycline inhibits microglial activation (Yrjanheikki et al., 1998), and in actuality it is one of the most frequently used drugs for inhibiting microglial activation (Yrjanheikki et al., 1999; Du et al., 2001; Kim and Suh, 2009). Several rodent studies have shown that stress increases microglial activation (Frank et al., 2007; Sugama et al., 2009; Tynan et al., 2010), and causes anxiety-like behaviors, which in turn can be decreased by minocycline treatment (Neigh et al., 2009). These *in vivo* studies have suggested that minocycline may be effective for the treatment of psychiatric disorders. An open-label study has shown that selective serotonin reuptake inhibitor (SSRI) and minocycline attenuate depressive and psychotic symptoms in patients with psychotic depression (Miyaoka et al., 2012). In addition, minocycline has been reported to be effective for the treatment of various symptoms in patients with Fragile X syndrome (FXS) such as social communication, anxiety, irritability, stereotypy, hyperactivity, and inappropriate speech (Paribello et al., 2010; Utari et al., 2010).

Previous microglia research has mainly highlighted the neuropathological aspects of microglia, while recent animal studies

have shown the normal functions of microglia (Graeber, 2010; Ransohoff and Stevens, 2011; Tremblay et al., 2011; Graeber and Christie, 2012; Schafer et al., 2012). Rodent microglia have been revealed to monitor synaptic reactions by having continuous direct contact with synapses not only in pathological brain but also in normal brain (Wake et al., 2009), and have proved to play essential roles in brain development such as in synaptic pruning (Paolicelli et al., 2011; Schafer et al., 2012). Therefore, even in normal conditions, microglia have been revealed to have some crucial roles in the homeostasis of synaptic conditions and in brain development. Moreover, we have recently reported that human social activities such as decision-making are modulated by minocycline not only in psychiatric patients but also in healthy persons (Kato et al., 2012; Watabe et al., 2012). Our human neuroeconomics studies have implied the possibility that brain development including neuron-microglia network establishment may formulate personality, and personality-oriented behaviors may be modulated by microglia (Kato et al., 2012). Thus, we suppose that microglia could be one of the crucial players in human mental development during early stages, and also in various social/mental activities after developmental stages including under healthy and pathological conditions beyond the neuron-synapse doctrine. A recent PET study has shown that minocycline inhibits microglial activation in humans (Dodel et al., 2010), thus we can prospect that minocycline may actively modulate human microglial activity.

Based on the above-mentioned reports, we hypothesize that microglia may be one of the bridging players between these highlighted neuroendocrine factors, normal/pathological mental conditions, and psychiatric disorders, while the underlying biological mechanisms have yet to be well considered. In addition, it is unknown whether microglia release neuroendocrine factors, while recent studies have suggested that these hormones affect microglia. Therefore, in this article, we would like to introduce and discuss the missing and possible link between the above-mentioned neuroendocrine factors, neuropsychiatric disorders, and microglia.

HYPOTHALAMIC-PITUITARY-ADRENAL AXIS-RELATED HORMONES

The HPA axis is the major stress response system which connects peripheral organs and the brain (Selye, 1950; Chrousos and Gold, 1992). In response to stress, corticotropin-releasing hormone (CRH) is secreted by the paraventricular nucleus (PVN) of the hypothalamus, and released into the pituitary gland, where CRH induces the release of adrenocorticotropic hormone (ACTH). Thereafter, ACTH activates the adrenocortical secretion of glucocorticoids. Finally, glucocorticoids suppress the release of CRH and ACTH as a negative feedback.

HPA AXIS AND PSYCHIATRIC DISORDERS

A variety of psychiatric symptoms due to primary physical illness are related to the HPA axis, such as the depressive symptoms associate with Cussing syndrome and steroid-induced depression (Lynn, 1995; Terao et al., 1997). The significant higher concentration of CRH and ACTH, induced by the initial injection of interferon (IFN)-α, has been found in patients with the subsequent development of depression during IFN-α treatment than in those without depression (Capuron et al., 2003).

The HPA axis has also been suggested to have a strong linkage to psychiatric disorders such as mood disorders and post-traumatic stress disorder (PTSD) (Baker et al., 1999; Holsboer, 2000; Kunugi et al., 2006). Baker et al. (1999) reported that CRH levels in cerebrospinal fluid were significantly greater in PTSD patients than in normal subjects. The dysregulation of the HPA axis is suggested to be involved with the pathophysiology of depression (Roy et al., 1988; Lesch et al., 1990). Previous studies have revealed the blunted responses to stressful events in patients with MDD, especially patients with a familial history of mood disorders and with men compared to women (Peeters et al., 2003). Investigations with depressed patients have shown that the number of CRH neurons increases in the PVN of the hypothalamus of patients with depression (Raadsheer et al., 1994), and pituitary volume decreases in patients with bipolar disorder (Sassi et al., 2001). ACTH has been reported to induce depressive-like behaviors through NMDA receptors in rats (Tokita et al., 2012). Glucocorticoids are also thought to be involved in various psychiatric disorders and related emotional disturbances (Brown, 2009; Laan et al., 2009; Ros-Bernal et al., 2011). Recently, Niwa et al. have reported the essential role of glucocorticoids in the association between adolescent stress and gene-environmental interactions using a mouse model with dominant-negative DISC1 (Disrupted Schizophrenia 1) under isolation during adolescence, and suggested this mouse as a candidate model for psychotic depression. They have revealed that via the epigenetic functions of glucocorticoids, adolescent stress induces projection, originating from the ventral tegmental area, specific methylation of tyrosine hydroxylase, and subsequently causes some neurochemical and behavioral deficits in this model mouse (Niwa et al., 2013). These reports have suggested that the HPA axis is one of the key components in understanding the deeper mechanisms of mood disorders and other psychiatric disorders.

CORTICOTROPIN-RELEASING HORMONE AND MICROGLIA

Corticotropin-releasing hormone, otherwise known as corticotropin-releasing factor (CRF), is a 41-amino acid peptide hormone, originally derived from a 191-amino acid preprohormone (Vale et al., 1981). The main site of CRH production is neurons in the parvocellular division of the hypothalamic PVN. CRH is also distributed in the limbic system such as other areas of the hypothalamus and amygdala, the locus ceruleus of the brain stem, A1, A5 catecholaminergic cell groups, and cerebral cortices (Chappell et al., 1986; Dunn et al., 2004; Chandrasekar et al., 2007). CRH mainly binds to CRH receptor I (CRH-R1). Urocortin, which also binds to CRH-R1, was discovered in rat midbrain (Vaughan et al., 1995), and is now characterized in three subtypes as urocortin-I/II/III (Lewis et al., 2001; Pelleymounter et al., 2004). Urocortins have high affinity to another CRH receptor; CRH receptor II (CRH-R2). Urocortin-II and urocortin-III specifically bind to CRH-R2. Urocortins are known to modulate various aspects; for example, appetite and anxiety in the brain, and the cardiovascular system in peripheral organs (Oki and Sasano, 2004).

Rat microglia have functional CRH-R1, and CRH has shown to bind to CRH-R1 (Wang et al., 2002), which results in microglial proliferation and TNF-α release via mitogen-activated protein kinase (MAPK) signaling pathways (Wang et al., 2003). On the

other hand, Ock et al. reported that CRH induced an apoptosis of mice microglia, and did not influence NO production or expression of pro-inflammatory genes, indicating that CRH did not affect the inflammatory activation of microglia. The CRH-induced microglial apoptosis has shown to involve a mitochondrial pathway and reactive oxygen species (ROS), based on the mitochondrial membrane potential change, caspase 9 activation, and sensitivity to antioxidants (Ock et al., 2006). These reports have suggested that CRH induces a part of pro-inflammatory reactions and/or oxidative stress, while further investigations are needed for a more detailed understanding.

Interleukin (IL)-18 has been suggested to be one of the crucial cytokines modulating stress responses (Tringali et al., 2005). Microglia is the major source of IL-18 in the brain (Prinz and Hanisch, 1999). Recent reports have suggested that IL-18 may play a significant role in psychiatric disorders such as depression and PTSD (Shirts et al., 2008; Mehta et al., 2011; Prossin et al., 2011; Xiu et al., 2012). Yang et al. (2005) reported that CRH enhanced IL-18 mRNA expression and significantly induced the secretion of functional IL-18 protein in mouse BV2 microglial cells. IL-18 knockout mice showed less microglial activation after acute stress, which resulted in less damage of dopamine neurons (Sugama et al., 2004, 2007). On the other hand, CRH increased the generation of intracellular reactive oxygen intermediates (ROI), and CRH-induced IL-18 production was blocked by an antioxidant, *N*-acetyl-L-cysteine (NAC) in mouse microglia (Yang et al., 2005). This report suggests that stress response is involved in regulating CRH-induced IL-18 production via ROI modification in microglia.

Meanwhile, microglia in rat/mice also have functional CRH-R2 receptors (Wang et al., 2007). Urocortin suppressed the release of TNF-α from lipopolysaccharide (LPS)-activated microglia via CRH-R2, and attenuated the LPS-induced neuronal damage on neuron-microglia mix culture (Wang et al., 2007). This report indicates the possibility that urocortin has neuroprotective properties via anti-inflammatory effects on microglia, similar to other immunological cells (Gonzalez-Rey et al., 2010). A recent study using a triple urocortin knockout mouse model has suggested that urocortins play an essential role in stress recovery (Neufeld-Cohen et al., 2010). The latest study has revealed that CRH and urocortin modulate spinal outgrowth and synaptic formation via CRH receptors (Gounko et al., 2013). Direct interactions between microglia and these functions of CRH and urocortins have not been investigated (Neufeld-Cohen et al., 2010; Gounko et al., 2013), while we suppose that microglia may play an important role.

GLUCOCORTICOIDS AND MICROGLIA

To our knowledge, a direct relationship between ACTH and microglia has not been reported. On the other hand, glucocorticoids, another key modulator of the HPA axis, are suggested to induce microglial modulation in the CNS (Sorrells et al., 2009). Previous studies have discussed the role of glucocorticoids as moderators of stress-related neuroinflammation (Dinkel et al., 2003; Munhoz et al., 2010; Loram et al., 2011). Smith et al. (1991) reported that adrenalectomy powerfully potentiates CNS inflammatory responses. Regarding microglia, Tanaka et al. (1997) have reported that cultured microglia isolated from the forebrain of newborn rats express glucocorticoid receptor in the plasma membrane. Sierra et al. (2008) have shown that corticosterone attenuated the production of TNF-α, IL-6, and NO from LPS + IFN-γ-activated murine microglia, which suggests that the anti-inflammatory effect of glucocorticoids on microglia is inverted to that of CRH. The stress-induced elevation of glucocorticoids has been well known (Munck et al., 1984), and it has proven to activate microglia in rats (Sugama et al., 2007; Tynan et al., 2010) and promote the proliferation of microglia via NMDA receptor activation in mice (Nair and Bonneau, 2006). From the temporal point of view it is important to categorize stress into two types; acute and chronic stress (Frank et al., 2010). Stress and administration of glucocorticoids prior to the injection of LPS, a peripheral immune stimuli, exerts pro-inflammatory effects on microglia in rats (de Pablos et al., 2006; Frank et al., 2010, 2012). On the contrary, exposure to glucocorticoids after LPS stimulation has anti-inflammatory properties in rats (Frank et al., 2010). In macrophage, glucocorticoids have been reported to exert pro-inflammatory effects through the increased NOD-like receptor (NLR) P3 mRNA and protein, which is a critical component of the inflammasome (Busillo et al., 2011). Deeper mechanisms of pro- and/or anti-inflammatory effects of glucocorticoids on microglia have not been well clarified, while a recent study has suggested that corticosteroids limit microglial activation occurring during acute stress when using adrenalectomy plus corticosterone administered rats (Sugama et al., 2013). Further investigations should be conducted to dig up the deeper mechanism.

CLINICAL IMPLICATIONS

The HPA axis has long been suggested to have strong linkage to psychiatric disorders, and the role of hormones such as CRH and glucocorticoids have been considered within the understanding of the HPA axis. Our highlighted evidence that CRH and glucocorticoids directly affect microglia sheds new light on understanding the unsolved roles of CRH and glucocorticoids in psychiatric disorders and psychopathologies. CRH and glucocorticoids directly and/or mutually modulate activities of microglia, and activated microglia release pro-inflammatory mediators, which may result in various psychiatric symptoms such as anxiety, fear, and depression beyond the classical understanding of the HPA axis (**Figure 1**). So far, the underlying mechanism of the mutual effects of CRH and glucocorticoids on microglia has not been well understood, while such novel knowledge will provide a systematic understanding of psychiatric disorders. Further basic, clinical and translational studies of CRH and glucocorticoids focusing on microglia should be investigated.

SEX HORMONES

Sex hormone system, frequently conceptualized as the hypothalamic-pituitary-gonadal (HPG) axis, is one major category of hormones correlated with mental conditions. Sex hormones such as estrogen, progesterone, and testosterone have crucial roles in health. Estrogen is one of the important steroid hormones and mainly classified into estrone, estradiol, and estriol. Androstenedione and testosterone metabolized from cholesterols in theca cells can be aromatized to estrone and estradiol in granulosa cells, respectively. Estradiol is also produced in various regions