Immunological and neuroimaging biomarkers of complicated grief

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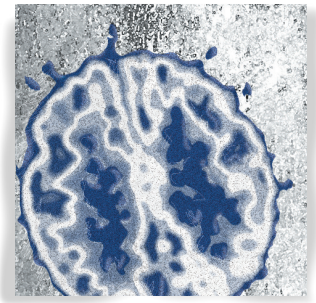

Complicated grief (CG) is a disorder marked by intense and persistent yearning for the deceased, in addition to other criteria. The present article reviews what is known about the immunologic and neuroimaging biomarkers of both acute grief and CG. Attachment theory and cognitive stress theory are reviewed as they pertain to bereavement, as is the biopsychosocial model of CG. Reduced immune cell function has been replicated in a variety of bereaved populations. The regional brain activation to grief cues frequently includes the dorsal anterior cingulate cortex and insula, and also the posterior cingulate cortex. Using theory to point to future research directions, we may eventually learn which biomarkers are helpful in predicting CG, and its treatment.

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Introduction

Complicated grief (CG) is a disorder of significant impact,[1] as described in other articles in the current issue. An important question with which psychiatrists, researchers, the *DSM-5* committee, and the general public have wrestled is how to address the unique suffering of those with CG, and how to distinguish it from acute grief, which may also cause difficult emotional reactions. The present article reviews what is known about the immunologic and neuroimaging biomarkers of both acute grief and CG.

Evidence from the past three decades has indicated that immunological changes occur in those who have experienced the death of a loved one, which may impact physical health. Newer evidence suggests which neural regions are activated in response to grief cues. Although only empirically defined as a disorder in the past two decades, recent research has compared CG with non-complicated grief (non-CG) to determine whether severity of grief may have greater explanatory power than the demographic category of bereaved/nonbereaved. The present article begins by reviewing theories that incorporate physiological aspects of general bereavement (ie, attachment theory and cognitive stress theory), and then reviews theories that incorporate physiological changes in CG specifically. Next, empirical evidence for the immunologic and neuroimaging aspects of bereavement, and of CG specifically, will be reviewed. Finally, the article ends with a summary of some of the gaps in knowledge of the neurobiological and immunological aspects of CG.

Bereavement models and theories

Why investigate the immunological and neuroimaging biomarkers of CG? Certainly there is value in the mere evidence of these biomarkers, but in addition, the physiological components or correlates of CG may help us to understand how CG arises, predict who it may affect, and provide suggestions for how to treat it. However, these latter reasons are best served when there is a clear theory behind the study of the biomarkers. Theory points us in the direction for study, and the results of the studies inform and refine our theories. The following section reviews cognitive stress theory, attachment theory, and the biopsychosocial model of CG.

Cognitive stress theory suggests that the death of a loved one is stressful because it is a disruptive event requiring a great deal of adjustment.[2] In addition, at exactly the moment when one must cope with a significant stressor, a primary source of support may be absent (ie, the deceased), reducing one's emotional and instrumental resources. This is one definition of stress: the perceived demands of the situation tax or exceed the individual's perceived coping resources.[3]

Attachment theory states that the bonds between parent and child, and romantic partners, is a product of behavioral conditioning whereby an association is developed between the attachment figure and: (i) a reduction in distress; and (ii) the generation of pleasure.[4] This conditioning explains a variety of behaviors, such as the maintenance of close proximity between bonded individuals, the development of mental schemas, or working models, that provide comfort during absence of the attachment figure, and distress that is generated upon separation from the attachment figure. For bereavement, attachment theory has specific predictions. Bereavement includes a gradual extinction of this conditioning, in which the regulatory benefits conferred by mental representations of the attachment figure diminish slowly over time. Bowlby[4] described the end point of successful mourning as a psychological reorganization of one's thoughts and feelings about a deceased attachment figure (for review, see ref 5).

In a very elegant study comparing cognitive stress theory and attachment theory, Stroebe and colleagues[2] examined a prospective dataset of older adults. At the baseline, both members of the couple were alive. At the second time point, one of the spouses had died. They hypothesized that attachment theory would predict that pre-bereavement marital quality would only affect yearning for the loved one who died, but not other more general grief reactions. Alternatively, cognitive stress theory would predict that support from family and friends, though unlikely to reduce yearning, might ameliorate general grief symptoms and depression. The results demonstrated that yearning was the only grief symptom associated with marital quality and was not associated with social support, consistent with predictions from attachment theory. Thus, although supportive others reduce depression and other general symptoms, they can not alleviate the loss of an attachment figure.

Physiological regulation

We can add to the original attachment theory (ie, that attachment confers capacity for psychological regulation) that it also may confer physiological regulation. Repeated social contact with a particular person results in a conditioned response whereby the attachment figure is reliably associated with a state of psychological security and physiological calm.[6] Much of the original work on physiological coregulation came from a series of studies by Myron Hofer.[7] These studies were designed to isolate different systems that became dysregulated when a rat pup was separated from its mother. For example, warmth and milk are two very different aspects of the loss. Hofer theorized that the diverse responses to loss could be understood in terms of the removal of "interpersonal regulators" which were physiological. He inferred that human bereavement also included the loss of physiological regulators, rather than only psychological stress.

Sbarra and Hazan[5] theorized that the response to separation (or bereavement) in fact has two unrelated (though usually co-occurring) physiological components. First, there is a general stress response (termed *organized* by Sbarra and Hazan). Second, there is an attachment-specific stress response (termed *disorganized* by Sbarra and Hazan) driven by the loss of the rewarding aspects of attachment.

First, bereavement provokes a general stress response—the physiological stress response that psychologists refer to as the "fight-or-flight" response, and includes the cardiovascular system (eg, heart rate, catecholamines) and the hypothalamic–pituitary–adrenal (HPA) axis (eg, corticotrophin-releasing hormone (CRH), cortisol). Bereavement research has demonstrated increases in

catecholamines and cortisol in the early stages of bereavement.[8-11] However, this general physiological stress response to bereavement is not distinct from the response to other stressful life events (eg, stress of job loss, stress associated with man-made disasters).

In addition to the general stress response, there is an attachment-specific stress response driven by the loss of the rewarding aspects of attachment.[12-14] Physiological systems respond to the removal of the conditioned pleasure and soothing associated with the attachment figure. Sbarra and Hazan[5] use the term "coregulation" to describe the physiological aspect of the feelings of security that an attachment figure provides.

The physiological systems responsible for this attachment-specific stress response include the dopamine system,[6] the opioid system,[15,16] and the oxytocin system.[17,18] The dopamine system is important in the experience of motivation to seek our rewards, both wanting to and, quite literally, moving toward a desired object. Dopamine is one of the neurotransmitters that is fundamental in conditioning, in associating the experience of reward with specific objects.[19] In the present discussion, this conditioning specifically creates the attachment to a *particular* figure. Dopamine is important in pursuing rewards, and opioids are important in the enjoyment of those rewards.[6] Opioids are another endogenous neurochemical, and they are also released in a variety of social interactions, including gentle physical touch. Oxytocin is a neurohormone important in birthing and nursing in all mammals, but in humans it has also been linked to suppression of anxiety during psychosocial stress and to the enhancement of trust.[20,21]

However, in order to explain why some individuals develop CG in response to the death of a loved one and others adjust resiliently, we must move beyond models and theories designed for bereavement generally. A biopsychosocial model of CG posits first that the symptoms of *acute* grief result from a temporary failure of biobehavioral regulatory functions resulting from the mental representation of the deceased person, much like what has been described above.[22] Acute grief resolves as the bereaved person assimilates the finality of the loss, and this knowledge is integrated into attachment-related long-term memory and mental schemas. This allows an effective attachment system to function again, and there is a reduction of overwhelming and intense sadness. Although acute grief is usually followed by resilient adjustment,[23] Shear and Shair[22] suggest that adjustment to the death may become complicated by maladaptive attitudes and behaviors (and perhaps new evidence will be discovered that includes physiological constraints of the neurobiological attachment system).

Creating a neurobiological model of CG faces a problem with the lack of evidence on a basic point. Does CG represent merely a person with acute grief whose process of adaptation has been interrupted, or does CG represent a wholly other process from noncomplicated bereavement adjustment? For example, CG may stem from a pre-existing individual difference, which is already present at the time of the death of the attachment figure. However, it may require the removal of the attachment figure for this pre-existing condition to be revealed in behavior.

Immunological biomarkers of grief

The effect of bereavement on the immune system has been empirically documented since the 1970s. Bartrop and colleagues[24] measured T-cell and B-cell functioning in widows at 2 weeks and 8 weeks following the death, and in controls. T-cell functioning was significantly reduced compared with controls, and was reduced at the second time point compared with the first. B cells showed no changes. These early results were theorized to occur because of the general stress response to such a distressing life event (as opposed to a grief-specific response).

Research on multiple aspects of immune functioning during bereavement continued through the 1980s,[25,26] the 1990s,[27-31] and the 2000s.[9,32,33] Generally, decreased natural killer cell cytotoxicity and poorer lymphocytic response to pathogens was found for bereaved individuals compared with nonbereaved individuals, and found particularly in early bereavement as compared with later bereavement. Of course, negative findings are less likely to be published, but overall, these findings are quite consistent. In addition, particular subpopulations have been studied because of their compromised immune status. A number of studies have investigated HIV-positive individuals, and their experience of the death of a partner.[28,29,34] In addition, bereaved older adults have been investigated, and they follow a similar pattern with the additional finding of reduced antibody titers to vaccination.[32] However, bereaved older adults have not demonstrated greater proinflammatory cytokines.[35]

Through this expansion of immunological research, the theoretical perspective primarily posited that bereave-

ment was an example of a nonspecific stressor (compared with other stressors such as space shuttle touchdown, significant illness of a spouse, insomnia, and other stressors summed from life event checklists). Additional moderators and mediators have been considered in different studies (eg, depressive disorder, active coping, finding meaning in the loss). All of these studies hypothesize that bereavement is a form of life stress, which although very severe, operates through known stress-response systems. To state this differently, the investigators presume that the distress leads to increases in the fight-or-flight response, and this leads to reduced cellular immune functioning. Regarding this general stress theory, some studies that investigated cortisol and immune parameters simultaneously and have not found changes in cortisol that could be linked to the immune decrements,[24,30,36] while others have found an association.[9,28,33]

One theory is that bereavement stress leads to depression, and only depressed bereaved persons show immune decrements. Several studies[26,31] found no immune functioning or immune population differences between bereaved and nonbereaved, but did find that widows who were depressed had lower natural killer cell activity and lower responsivity to mitogen stimulation than widows who were not depressed. Nonetheless, none of the authors suggest that there is an immune response that is specific to bereavement stress, but rather that bereavement is one example of the general stress response.

None of these studies have used a diagnosis of CG to shed light on who has immune impairment and HPA dysregulation in response to a death event. A recent study, however, compared the diurnal cortisol slope between those with CG and non-CG.[37] Participants provided saliva samples for 3 days, four times per day, to capture the diurnal rhythm. Exclusionary criteria included diagnosis with major depressive disorder and antidepressant use. Controlling for body mass index, the CG group showed a significantly flatter slope than those with non-CG. Perhaps CG as a disorder will be better able to predict grief-specific stress responses in cortisol than the dichotomous category of bereaved/nonbereaved, or than depressive disorder, although this will require additional research.

Neuroimaging biomarkers of grief

The initial neuroimaging study of bereavement used personalized stimuli to evoke grief.[38] A total of eight women who had experienced the death of a first-degree relative in the past year participated. Participants each provided a photograph of their deceased loved one, which was matched with a photo of a stranger on characteristics such as gender, age, indoor vs outdoor setting, snapshot vs portrait type of photograph. Grief-related words were taken from an interview of the participants about the death event (eg, collapse, funeral, loss) and were matched with neutral words (eg, announce, ceiling, list). These words were embedded into the photos to create composites. These picture-word composites resulted in a 2 x 2 factorial design with two routes of eliciting grief.

Behavioral results of the study included higher ratings of grief for the deceased with the grief word than the stranger, and electrodermal responses taken during scanning indicated that greater autonomic responsiveness to the pictures of the deceased as well. Regional neural activations that occurred in response to the pictures included, among other regions, the dorsal anterior cingulate cortex (dACC) and the insula. These regions are activated together in a range of studies examining both physical pain[39] and social pain, such as grief and rejection.[40,41] In addition, the posterior cingulate cortex (PCC) was activated during grief elicited both by the photos and the words. This region is involved in evaluating whether environmental stimuli are relevant to the self, particularly related to emotional memories.

Two additional functional neuroimaging studies have investigated acute bereavement.[42,43] In one study, 12 women who had experienced the loss of an unborn child in the past 2 months were compared with 12 women who had delivered a healthy child. The stimuli included unfamiliar babies with happy facial expressions and unfamiliar adults with happy and neutral facial expressions. By using unfamiliar baby faces as emotional cues in both groups, any contributions to grief-related activations other than the subjective experience of grief (such as the possibility of familiarity in the prior study by Gündel and colleagues) were avoided. Kersting and colleagues[42] hypothesized that the regions involved in social pain would be activated, and supporting this hypothesis, increased activity in the dACC and periaqueductal gray was observed for happy baby faces in bereaved women (vs controls). They also observed an increased activation in the PCC during the processing of happy baby faces in bereaved women (vs controls).

A second study focused on the neural correlates of the regulation of grief.[43] Acute grief due to the loss of a pet

was investigated through the use of grief-related vs familiar words in an emotional Stroop task. Grief symptoms of intrusive thoughts and self-reported avoidance were negatively correlated with functional connectivity between the amygdala and emotion regulatory regions (the rostral anterior cingulate cortex and the dorsolateral prefrontal cortex). PCC was also activated in response to grief-related words.

The importance of activation in the PCC has been clear since the first functional magnetic resonance imaging (fMRI) grief study. This area was activated both through word and photo grief cues. The additional two studies, with different types of grief-related stimuli, also had significant activation in this region. In other human and animal studies, this region is activated in autobiographical and emotional memory. Dense projections extend to the parahippocampal gyrus, making the role of the PCC in emotional memory anatomically likely. PCC activation is important during learning, and a recent review theorized:

> We predict that [PCC] activity will be more strongly modulated by new cues that predict environmental changes that require a cognitive set switch...Together, these observations indicate a healthy [PCC] is necessary for organizing flexible behavior in response to an ever-changing environment by mediating learning, memory, control, and reward systems to promote adaptive behavior.[44]

It is difficult to imagine a situation of greater personal relevance for an environmental change than learning to adapt to the death of a loved one. To this point, although it is unclear what longitudinal changes in functional activation may occur across adaptation, it is reasonable to hypothesize that PCC activation would be greatest during the period when a person is most actively accommodating the reality of the loss. For most bereaved persons, this would be early in the bereavement process.

In addition to the investigation of neural activation in general bereavement, one study has examined the neural response in those with CG.[45] If CG is a distinct phenomenon from non-CG, there should be differences in the neural activation. The participants included 11 women with CG and 12 women with non-CG. Exclusion criteria included Axis I psychiatric disorders (including current depression) and medical disorders. Analyses of the whole group (N =23) demonstrated activation in pain-related regions (eg, dACC, insula, and periaqueductal gray) in response to pictures of the deceased (vs a stranger).

Between groups, analyses revealed that CG participants showed greater activation compared with those with non-CG in a subcortical area of the brain, the nucleus accumbens, in response to reminders of the deceased. Research on both animals and humans clearly demonstrates that the nucleus accumbens is active during the processing of rewards. Reward can be decomposed into "wanting" and "liking," and elegant experimental designs have shown that the nucleus accumbens is activated when a reward is "wanted." [46] It is the reinforcement value of the reward that is associated with nucleus accumbens activation, and not the experiential aspect of reward. To clarify, the conscious level of processing may or may not include the feeling of reward, even when an object is reinforced.

In addition, correlational analyses were conducted between behavioral responses and activation in this region. Activation in the nucleus accumbens was not correlated with the amount of time that had passed since the death event, the participant's age, or the self-reported positive/negative affect after the scan. The nucleus accumbens activation was positively correlated with self-reported yearning at an interview in the week prior to the scan.

This result does not indicate that the nucleus accumbens activation is causal in distinguishing CG and non-CG (ie, this region does not necessarily cause impaired adaptation during grief, as its higher level of activation may be a consequence of the symptoms of CG). It also doesn't tell us if the region is related to individual differences, or whether its activation changes in intensity across adaptation. In other words, at least two possibilities exist: (i) those with CG would show distinctive activation in this region as an individual difference—perhaps even before the loss of a loved one; (ii) all individuals may show greater activation in this region early in adaptation to a loved one's death, and decreasing activation in this region as they adapt psychologically. In order to choose between these two explanations, future research must include multiple scans longitudinally, in order to observe change during adaptation.

Finally, it is not possible to know from functional neuroimaging what neurons in the nucleus accumbens region are the sources of this increased activation. For example, this brain region is rich in oxytocin, opioid, and dopamine receptors, and neurons that use one, two, or all three of these neurotransmitters may have been more active in those with CG than those with non-CG. Thus, future research using positron emission tomography

(PET), which quantifies the levels of these neurotransmitters in the central nervous system, would be a productive avenue of research in discriminating CG from non-CG.

For those with CG, reminders of the deceased activated neural reward activity, and this neural reinforcement may interfere with adapting to the loss in the present. Or, the nucleus accumbens activation may simply be a neurobiological indicator of where the bereaved is in the adaptation process. Because activation of this region is also seen in fMRI studies of adults viewing photos of their living romantic partners and their children,[47] it is reasonable to hypothesize that those with CG are responding subcortically to the cue because of the attachment relationship.

Conclusion

Why focus on the immunologic and neuroimaging biomarkers? One reason is precisely because these physiological variables may shed light on the similarities and differences between acute grief and CG. Along the same lines, studying the underlying aspects of the body's stress response to a death event may reveal distinctions between CG and post-traumatic stress disorder (PTSD), or CG and major depressive disorder. A second reason to focus on biomarkers is to generate theories as to how the death of a loved one can lead to the "broken-heart phenomenon," or the unexpected death of a recently bereaved individual. Given that morbidity and mortality are necessarily physical events, some interaction is occurring between the individual's knowledge of the loss and their physical body, and although the mechanisms linking them are not well understood, the immune system is a likely suspect. A third reason to focus on biomarkers is that understanding the mechanisms of CG may lead to improved treatment for this disorder. Although pharmacological treatment seems the obvious way to use biomarkers, psychological treatment that takes advantage of biomarkers is also possible. To draw on an example from another disorder, psychotherapy for PTSD has taken advantage of the discovery that when a patient's heart rate is high at the beginning of the first exposure treatment, therapy outcomes are better.[48]

The study of the physiology and neurobiology of CG is only at the earliest beginning. Self-regulation, at the psychological as well as physiological levels, may be important in coping with pangs of grief and in acceptance of the death of a loved one. The assimilation of the reality of the death occurs in the brain for the working model of attachment to be revised. One hypothesis is that if the assimilation of the new information does not occur, either for psychological reasons (eg, extreme guilt or avoidance) and/or biological ones (eg, the effect of flattened diurnal cortisol on hippocampal function), then the adaptation to the death may be prolonged and lead to CG.

Some physiological markers of CG will correlate with a separation distress response and others will correlate with a general stress response. The physiological markers that correlate with a general stress response may occur with other stressful life events, but the physiological markers that correlate with the separation distress should be specific to the loss of an attachment figure. In addition, the physiological markers correlated specifically to the loss of an attachment figure may be preexisting traits (endemic to the individual or to the relationship), or these physiological markers may develop, or fail to recover, across time during the adaptation process. If CG symptoms are mediated by attachment,[49] then understanding the neurobiology of attachment will no doubt assist in treating the CG response to bereavement. Observing and documenting the physiological response to bereavement, and how it shapes and is shaped by the psychological response, may help us to improve adaptation even in the face of one of life's most stressful events.

It is highly unlikely that there is a one-to-one correspondence between any particular physiological or neurobiological marker and CG. For one thing, physiological systems are part of a cascade and feed back information to each other, and therefore any single biomarker impacts a host of other biomarkers. As with biomarkers in most affective disorders, there are none that are ready to be used in a clinical setting to aid in diagnosis of CG yet. However, by measuring these markers, we may see what contributes to poor adaptation or what the physiological predictors of CG are. Using immunological and neuroimaging variables in bereavement research as one part of a multimethod approach will only increase our understanding of these phenomena. ❑

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Biomarcadores inmunes y neuroimágenes del duelo complicado

El duelo complicado (DC) es un trastorno caracterizado por la añoranza intensa y persistente por el fallecido, además de otros criterios. Este artículo revisa lo que se sabe acerca de los marcadores inmunes y las neuroimágenes en el duelo agudo y en el DC. Se revisan las teorías del apego y del estrés cognitivo en cuanto ellas son parte del duelo, así como lo es el modelo biopsicosocial en el DC. La reducción de la función de las células inmunes se ha replicado en diversas poblaciones de deudos. La activación cerebral regional en situaciones de duelo frecuentemente incluye la corteza cingulada dorsal anterior y la ínsula, y también la corteza cingulada posterior. A partir de la teoría que oriente la dirección de las futuras investigaciones, eventualmente se podrán conocer los biomarcadores útiles para predecir el DC y su tratamiento.

Biomarqueurs immunologiques et de neuro-imagerie du deuil compliqué

Le deuil compliqué est marqué par une aspiration intense et persistante pour le(a) décédé(e) s'ajoutant à d'autres critères. Cet article passe en revue les connaissances actuelles sur les marqueurs immunologiques et de neuro-imagerie dans le deuil aigu et compliqué. Les théories de l'attachement et du stress cognitif sont analysées dans le cadre du deuil, comme l'est également le modèle biopsychosocial de deuil compliqué. Une fonction cellulaire immunitaire diminuée a été reproduite dans des populations endeuillées différentes. L'activation cérébrale régionale en réponse aux stimuli rappelant le deuil concerne souvent le cortex cingulaire dorsal antérieur et l'insula ainsi que le cortex cingulaire postérieur. En regardant vers la recherche future, nous apprendrons un jour quels biomarqueurs pourront aider à prédire un deuil compliqué et son traitement.

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