Neuroimaging in Functional Neurological Disorder: State of the Field and Research Agenda

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1. Introduction

Functional neurological disorder (FND), also known as conversion disorder and previously termed hysteria, is a prevalent and disabling condition at the interface of neurology and psychiatry. During the 20th century, neurology and psychiatry grew apart—leaving FND a borderline condition. Fortunately, a renaissance has occurred in the last two decades, fostered by increased recognition that FND is prevalent and diagnosed using “rule-in” examination signs. The parallel use of scientific tools to bridge brain structure-function relationships has helped refine an integrated biopsychosocial framework through which to conceptualize FND. In particular, a growing number of quality neuroimaging studies using a variety of methodologies have shed light on the emerging pathophysiology of FND. This renewed scientific interest has occurred in parallel with enhanced interdisciplinary collaborations, as illustrated by new care models combining psychological and physical therapies and the creation of a new multidisciplinary FND society supporting knowledge dissemination in the field. Within this context, this article summarizes the output of the first International FND Neuroimaging Workgroup meeting, held virtually, on June 17th, 2020 to appraise the state of neuroimaging research in the field and to catalyze large-scale collaborations. We first briefly summarize neural circuit models of FND, and then detail the research approaches used to date in FND within core content areas: cohort characterization; control group considerations; task-based functional neuroimaging; resting-state networks; structural neuroimaging; biomarkers of symptom severity and risk of illness; and predictors of treatment response and prognosis. Lastly, we outline a neuroimaging-focused research agenda to elucidate the pathophysiology of FND and aid the development of novel biologically and psychologically-informed treatments.
Given phenotypic heterogeneity, interpretation of FND-related neuroimaging findings depends a great deal on rigorous cohort characterization (Gelauff et al., 2020; Matin et al., 2017; McKenzie et al., 2011). Additionally, FND presents diagnostic challenges that likely impact neuroimaging research. These reflect, in part, the somewhat under-developed diagnostic criteria for “Conversion Disorder (Functional Neurological Symptom Disorder)” found in the Diagnostic and Statistical Manual of Mental Disorders—Fifth Edition (DSM-5) and International Classification of Diseases–11th Revision (ICD-11) (Stone et al., 2014). In DSM-5, a patient can be diagnosed with FND if they have motor and/or
sense findings providing “evidence of incompatibility between the symptom and recognized neurological or medical conditions” (American Psychiatric Association, 2013; Stone et al., 2010b). The symptom must impair social and/or occupational functioning or lead individuals to seek a medical opinion. There are no duration or severity criteria or explicit rules for exclusion based on additional symptoms. In the neurological literature, there are also diagnostic criteria for FND sub-types, such as those for FND-seiz (LaFrance et al., 2013a) and FND-movt (Espay and Lang, 2015; Gasca-Salas and Lang, 2016; Williams et al., 1995). Unsurprisingly, varying FND diagnostic criteria have been used across studies. Despite this heterogeneity, a general emphasis on “rule-in” examination signs and seminal features guiding diagnosis has been used, including the ‘gold-standard’ adjunctive video-electroencephalography (vEEG) for FND-seiz (LaFrance et al., 2013a).

Despite these potential concerns with the current criteria, FND diagnoses are reliable over time - misdiagnosis is rare in published studies (Stone et al., 2005). The same cohort studies in adults also show symptom persistence in the majority, indicating that we are generally not dealing with transient symptoms, at least in research populations where participants have usually been ill for months to years before inclusion (Gelauff and Stone, 2016).

Based on phenotypic complexity, FND neuroimaging researchers need to consider how the following factors could lead to heterogeneity:

a. **Symptom severity:** For example, we likely want to differentially consider a patient with a heavy leg that drags occasionally and someone with quadriplegia.

b. **Episodic vs. persistent:** Whilst some symptoms are episodic such as seizures, others may vary. Patients may at times have only intermittent limb weakness, and at other instances a more static paralysis.

c. **Duration and Onset:** It is likely that someone who has had functional paraplegia for 20 years would have different neuroimaging correlates compared to an individual with symptoms for a month (including compensatory neuromodulatory changes from underuse) (Newbould et al., 2020). Illness duration may be especially important in connectivity and structural neuroimaging studies. Additionally, FND neural mechanisms in children/adolescents, adults and late-life presentations require inquiry regarding to what extent mechanisms are shared across the lifespan.

d. **Symptom type and overlap:** Many patients with FND have overlapping symptoms. For example, some with functional tremor or dystonia will also exhibit functional weakness or sensory deficits in the same limb and/or have current or past FND-seiz (Gelauff et al., 2020; Matin et al., 2017).

e. **Symptom location:** It may be important for some studies to explicitly define symptom location, for example, face, arm or leg, and the laterality.

Additional physical and mental health diagnoses in FND, at least as currently defined, are the norm rather than an exception (Nicholson et al., 2020). Pain, fatigue, insomnia, and cognitive symptoms are generally found in more patients with FND than not. Other functional somatic disorders such as functional bowel, bladder and cardiorespiratory symptoms are also common. Psychiatric conditions, especially affective, trauma-related and/or dissociative disorders, are typically present in over 50% of most FND samples, with lifetime rates even higher (Bowman and Markand, 1996; Goldstein et al., 2020; Gray et al., 2020; Mazewski et al., 2011; Kranich et al., 2011; Sar et al., 2004; Stone et al., 2010c). Personality disorders also exist in patients with FND at higher frequencies than that in the general population, with personality traits such as neuroticism, obsessiveness and/or emotional dysregulation commonly recognized (Ekayake et al., 2017; Szalarski et al., 2015). Adverse life events, a predisposing vulnerability for FND, are frequently reported though not universally so (Ludwig et al., 2018).

Additionally, other neurological conditions may be present, such as patients with both FND-seiz and epileptic seizures, or individuals with Parkinson’s disease and motor FND (Kutluabaei et al., 2018; Wissel et al., 2018); the intersection of mild traumatic brain injury and FND-seiz is also well recognized (LaFrance et al., 2013b; Popkirov et al., 2018). Psychotropic medications are frequently prescribed in patients with FND to manage anxiety, depression, pain, fatigue, and insomnia, among other symptoms. In FND, neuroimaging studies have varied in their characterization of other concurrently present medical/neurological and psychiatric conditions; for example, only a subset of studies performed structured psychiatric interviews. Additionally, only a few studies have attempted to adjust for psychiatric symptoms and medication effects in their analyses. Relatedly, serotoninergic-based medications modulate emotion processing circuits (particularly the amygdala), and efforts to adjust for medication use may help reconcile differences in findings across studies (Godlewksa et al., 2012).

How is the neuroimaging researcher to deal with this complexity and potential confounding? Control group considerations discussed below are one approach. Studying FND severity using within-group designs may also be helpful but complex, as studies show that symptom severity and affective symptoms can co-vary (Rawlings et al., 2017). Additionally, there are difficulties in knowing whether to attempt objective measurements or to rely entirely on patient reports. A recent international collaboration on FND outcome measures concluded that measures based on subjective reports were probably more meaningful, especially when considering that symptoms like functional leg weakness, by definition, can often be demonstrated temporarily to be absent (Nicholson et al., 2020; Pick et al., 2020). Similarly, regarding risk factors, subjective reports of high childhood maltreatment burden are strong predictors of later-life psychopathology - irrespective of objective documentation (Danese and Widom, 2020). Although large samples are required to avoid type 2 errors, another solution includes using statistical adjustments for possible confounding factors in secondary analyses (e.g., performing analyses and reporting findings adjusting and not adjusting for variables such as depression scores). A more radical perspective is to reframe some of the diagnostic and comorbidity challenges by adopting a position that FND, in its naturally presenting state, is not a pure disorder, and its ‘comorbidities’ are intrinsic to its pathophysiology. Those seeking to study FND should therefore potentially not regard prevalent ‘comorbidities’ as noise / nuisance factors, but part of the condition to be understood.

4. Control Group Considerations

The choice of controls is a key design element for FND studies. In FND, the majority of studies to date have used healthy controls or within-subject designs (Allendorfer et al., 2019; Bégue et al., 2019; McSweeney et al., 2017; Voon et al., 2016). Using healthy controls has benefits, including that between-group findings can be established to be outside the normal range; however, covarying affective symptoms and psychotropic medication use in the FND cohort limit the ability to relate observations as definitively associated with FND itself.

Another option is to use patient controls with comparable non-FND symptoms to those found in the FND cohort. Only a few studies have used neurological or psychiatric controls in FND research to date, including primary dystonia (Espay et al., 2018b; Schrag et al., 2013), essential tremor (Espay et al., 2018c), traumatic brain injury (Balandran et al., 2020; Goodman et al., 2020) and a mixed depression/anxiety psychiatric control group (Dziek et al., 2020). However, neurological and psychiatric control groups are inherently abnormal, and therefore attributing findings solely to the FND group can be challenging. Including two controls groups (one healthy and one neurological control group) may be preferable for many study designs (Espay et al., 2018b, 2018c; Szalarski et al., 2018); it may be particularly useful when the choice of the neuropsychiatric control group enables the subtraction out of effects related to conditions commonly co-occurring in FND (e.g., chronic migraine, generalized anxiety disorder, personality disorder, etc.), while the parallel use of healthy controls
contextualize findings as outside or inside the range of normal. While a transdiagnostic approach embracing the recruitment of mixed FND cohorts is increasingly being adopted to aid the investigation of shared neural mechanisms across subpopulations (Perez et al., 2015), including two or more isolated FND subtypes (e.g., functional limb weakness vs. functional dystonia) may aid the identification of subtype-specific findings (Canu et al., 2020; Sojka et al., 2021; Tomic et al., 2018). Relatedly, initial machine learning neuroimaging studies investigating the utility of such approaches as adjunctive diagnostic tools used healthy controls (Vasta et al., 2018; Wegrzyk et al., 2018), but including conditions on the differential diagnosis for FND (e.g., epilepsy, primary dystonia) will further test the specificity of such methods.

In terms of state (patients with active symptoms) vs. trait (patient-specific characteristics in those without current symptoms), within-group longitudinal designs can be informative. Some studies used patients as their own controls, with and without symptoms (Vuilleumier et al., 2001) or before and after treatment (Espay et al., 2019; Faul et al., 2020). An advantage of within-subject designs are their enhanced statistical power.

Note: task organization in this table aids to broadly group paradigms across studies based on similar constructs tested, however, the reader should note that there are important nuances to many of these tasks that should be carefully inspected by reading the original article. Abbreviations: FND, functional neurological disorder; FND-par, functional limb weakness/paresis; FND-movt, functional movement disorder; motor FND includes both FND-movt and FND-par; FND-3PD, persistent postural perceptual dizziness; FND-sensory, FND with sensory symptoms; TBI, traumatic brain injury.

Table 1
Examples of task-based neuroimaging studies in functional neurological disorders.

<table>
<thead>
<tr>
<th>Tasks</th>
<th>Task Descriptions</th>
<th>Construct(s) Interrogated</th>
<th>FND Type</th>
<th>FND Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motor-related</td>
<td>Preparing and attempting to move limbs</td>
<td>Motor preparation, performance, observation, control, and/or imagery</td>
<td>FND-par</td>
<td>Marshall et al., 1997</td>
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<tr>
<td></td>
<td>Joystick paced movements</td>
<td></td>
<td>FND-par</td>
<td>Spence et al., 2000</td>
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<td></td>
<td>Preparing and attempting projected hand movements</td>
<td></td>
<td>FND-par</td>
<td>Burgmer et al., 2006</td>
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<td></td>
<td>Action choice based on visual stimuli</td>
<td></td>
<td>FND-par</td>
<td>Stone et al., 2007</td>
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<td></td>
<td>Judging laterality of visually presented rotated hands</td>
<td></td>
<td>FND-par</td>
<td>de Lange et al., 2007</td>
</tr>
<tr>
<td></td>
<td>Go / No-Go task</td>
<td></td>
<td>FND-par</td>
<td>Cojan et al., 2009</td>
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<tr>
<td></td>
<td>Imagination and execution of movements</td>
<td></td>
<td>FND-par</td>
<td>van Beilen et al., 2011</td>
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<td></td>
<td>Action selection task</td>
<td></td>
<td>FND-movt</td>
<td>Voon et al., 2011</td>
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<tr>
<td></td>
<td>Metronome paced movements</td>
<td></td>
<td>FND-dystonia</td>
<td>Schrag et al., 2013</td>
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<tr>
<td></td>
<td>Passive movements of hands</td>
<td></td>
<td>FND-par</td>
<td>Hassa et al., 2017</td>
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<tr>
<td></td>
<td>Finger tapping task</td>
<td></td>
<td>FND-dystonia</td>
<td>Espay et al., 2018b</td>
</tr>
<tr>
<td></td>
<td>Finger tapping task</td>
<td></td>
<td>FND-tremor</td>
<td>Espay et al., 2018c</td>
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<tr>
<td>Affective and threat processing</td>
<td>Facial emotion recognition</td>
<td>Affective processing &amp; control, traumatic memory processing, avoidance learning, and/or psychological stress response</td>
<td>FND-movt</td>
<td>Voon et al., 2010a</td>
</tr>
<tr>
<td></td>
<td>Motivational and conditioned associative learning</td>
<td></td>
<td>Motor FND</td>
<td>Aybek et al., 2015</td>
</tr>
<tr>
<td></td>
<td>Easy vs. hard math and positive vs. negative social feedback (Montreal Stress Imaging Task)</td>
<td></td>
<td>FND-par</td>
<td>Hassa et al., 2017</td>
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<tr>
<td></td>
<td>Viewing emotive images</td>
<td></td>
<td>FND-seiz</td>
<td>Espay et al., 2018b</td>
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<td></td>
<td>Recall of trauma-themed events with varying relevance to symptom onset</td>
<td></td>
<td>FND-tremor</td>
<td>Espay et al., 2018c</td>
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<tr>
<td></td>
<td>Affectively conditioned associative learning</td>
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<td>Finger tapping task</td>
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<td>Espay et al., 2018c</td>
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<td></td>
<td>Finger tapping task</td>
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<td>FND-mixed</td>
<td>Morris et al., 2017</td>
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<tr>
<td></td>
<td>Recall of trauma-themed events with varying relevance to symptom onset</td>
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<td>FND-seiz</td>
<td>Allendorfer et al., 2019</td>
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<td></td>
<td>Functional vs. voluntary tremor task</td>
<td></td>
<td>FND-movt (TBI)</td>
<td>Balachandran et al., 2020</td>
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<td></td>
<td>Glove-based hand motion control</td>
<td></td>
<td>Motor FND</td>
<td>Aybek et al., 2010b</td>
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<td></td>
<td>Libet Clock</td>
<td></td>
<td>FND-mixed</td>
<td>Baek et al., 2017</td>
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<tr>
<td>Emotional-motor interaction</td>
<td>Grip force measure while observing emotional images</td>
<td>Limbic-motor interactions</td>
<td>FND-par</td>
<td>Hassa et al., 2017</td>
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<td></td>
<td>Passive movement while observing emotional faces</td>
<td></td>
<td>FND-movt</td>
<td>Faul et al., 2020</td>
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<td></td>
<td>Emotional Go / No-Go</td>
<td></td>
<td>FND-par</td>
<td>Hassa et al., 2017</td>
</tr>
<tr>
<td>Somatosensory perception</td>
<td>Vibro-tactile stimuli application</td>
<td>Sensory processing</td>
<td>FND-par</td>
<td>Vuilleumier et al., 2001</td>
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<tr>
<td></td>
<td>Brush stimulation</td>
<td></td>
<td>FND-sensory</td>
<td>Ghaffar et al., 2006</td>
</tr>
<tr>
<td>Other paradigms</td>
<td>Intense mechanical stimulation</td>
<td>Pain processing</td>
<td>FND-sensory</td>
<td>Burke et al., 2014</td>
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<tr>
<td></td>
<td>Virtual-reality rollercoaster stimulation</td>
<td>Self-motion perception</td>
<td>FND-3PD</td>
<td>Mailis-Gagnon et al., 2003</td>
</tr>
<tr>
<td></td>
<td>Visually-guided action judgement using perceptual guidance</td>
<td>Metacognition &amp; motor awareness</td>
<td>FND-3PD</td>
<td>Riccielli et al., 2017</td>
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<tr>
<td></td>
<td>Motor FND</td>
<td></td>
<td>FND-3PD</td>
<td>Passamonti et al., 2018</td>
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<td></td>
<td>Motor FND</td>
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<td>Motor FND</td>
<td>Bégue et al., 2018</td>
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</table>
5. Task-based Neuroimaging

Task-based functional MRI (fMRI) measuring blood-oxygen-level-dependent (BOLD) signal, and to a lesser extent nuclear imaging approaches (i.e., positron emission tomography (PET), single-photon emission computed tomography (SPECT)) has been used to interrogate neural activations in FND. Examples of experimental paradigms used to date are illustrated below and in Table 1.

Initial studies explored motor execution/control circuits in patients with functional limb weakness while participants tried to move their parietic limb compared with their unaffected limb (Marshall et al., 1997), or compared with subjects feigning limb paralysis (Spence et al., 2000; Stone et al., 2007). Other early functional limb weakness case studies probed motor preparedness, motor observation and imagined movements (Burgner et al., 2006; Cohan et al., 2009; de Lange et al., 2007; Marshall et al., 1997; van Beilen et al., 2011). Basic motor performance (e.g., finger tapping) tasks have also been used in FND-movt populations (Espay et al., 2018b, 2018c; Schrag et al., 2013). Recent studies have investigated the interplay between motor control and emotion processing using paradigms such as an emotional Go/No-Go (Paul et al., 2020; Hassa et al., 2017); in another study, participants were asked to maintain grip strength while viewing affectively-valenced images (Blakemore et al., 2016).

Limbic and salience networks can be interrogated with affectively-valenced facial expression processing tasks, an approach commonly used in FND research (Aybek et al., 2015; Hassa et al., 2017; Voon et al., 2010a). Other studies used affectively-valenced picture viewing tasks (e.g., the International Affective Picture System) to probe emotion processing/regulation circuits in FND populations (Espay et al., 2018b, 2018c; Sojka et al., 2019). Using a different approach, an affective memory response was explored by invoking traumatic memories with varying theorized relevance to patients’ functional neurological symptoms in the scanner (Aybek et al., 2014b; Kanaan et al., 2007). Cognitive-mediated stress responses in FND have also been investigated using easy vs. hard mathematical calculations and positive vs. negative social stressors from the Montreal Stress Imaging Task (Allendorfer et al., 2019; Balachandran et al., 2020).

Another construct explored is impaired self-agency in patients with FND-movt perceiving their functional movements as involuntary (Bayzel-Carvallo et al., 2019). One study compared brain activations during two conditions where the voluntary perception of tremor was different: in one condition patients could voluntarily trigger a tremor by moving their arm into a certain position and in the other condition patients displayed their functional tremor without any perceived voluntary control (Voon et al., 2010b). Other studies have interrogated self-agency using the Libet clock paradigm (Baek et al., 2017) and by manipulating visual feedback of a motor action with a cyber glove (Nahab et al., 2017).

Additional constructs probed in FND task-based neuroimaging research to date include avoidance learning (Morris et al., 2017), motor inhibition (Cohan et al., 2009), virtual-reality associated motor perception (Riccielli et al., 2017), meta-cognition (Bégue et al., 2018), symptom modeling with suggestion (Deeley, 2016), somatosensory processing(Burke et al., 2014; Ghaffar et al., 2006; Vuilleumier et al., 2001) and nociception (Mailis-Gagnon et al., 2005). No functional neuroimaging studies to date have explicitly probed catastrophizing, interoception or predictive processing more broadly, which are additional constructs of theoretical relevance to the pathophysiology of FND (Drane et al., 2020; Edwards et al., 2012; Fabian et al., 2020; Korokl et al., 2020). Relationships between lateralized symptoms and hemispheric brain activations also require additional inquiry, including the use of flipped and unflipped data analytic approaches (van Beilen et al., 2011).

For task-based neuroimaging, there are several relevant methodological considerations. Block designs favored in many early FND studies have superior statistical power but can yield confounds arising from stimulus order predictability (Friston et al., 1999). Event-related designs, while lacking somewhat in statistical power, allow sorting of trial responses according to specific behavioral outcomes such as motor errors or a subjective judgment of affectively-valenced stimuli (Chee et al., 2003). Although task fMRI has good spatial resolution (compared to PET and SPECT), temporal resolution is limited by the hemodynamic response, which is a concern when studying sub-second range cognitive-affective-perceptual processes (Khanna et al., 2015). Task fMRI techniques in-development utilizing fast, sub-second approaches may soon overcome these issues (Sahib et al., 2018).

6. Resting State Networks

RsfcMRI measures BOLD signal while an individual is awake but not engaged in any specified task. To analyze BOLD signal in the resting-state, some FND researchers have studied the temporal dynamics of the time series (e.g., amplitude of low frequency fluctuations (ALFF)) (Li et al., 2015a; Yang et al., 2020). However, rsfMRI approaches interrogating brain networks in FND cohorts are more popular (Chen et al., 2020; Foroughi et al., 2020) (see Table 2). rsfMRI analyses examine the dependency between the time series of different brain regions, computing a similarity measure between them. A common connectivity measure used in FND research is seed-based functional connectivity that characterizes relationships between a seed (region-of-interest (ROI)) and the rest of the brain. In FND studies, seed selection has been based on task fMRI activations within the same cohort (Allendorfer et al., 2019; Baek et al., 2017; Szafarski et al., 2018; van der Kruisj et al., 2012), neuroanatomical atlases (e.g., automatic anatomic labeling) (Canu et al., 2020; Lee et al., 2018; Li et al., 2015b; Morris et al., 2017), voxel-based coordinates informed by meta-analyses (Canu et al., 2020; Maurer et al., 2016), or a priori hypotheses (Spagolo et al., 2020). While seed-based connectivity is readily interpretable, this approach does not necessarily capture a complete picture of the global brain architecture. To reduce the dimensionality of whole brain data into a set of networks, studies in FND have employed data-driven parcellations including independent component analysis (ICA) (Canu et al., 2020; van der Kruisj et al., 2014) and clustering (Monsa et al., 2018). To study intrinsic network architecture properties, graph theory rsfMRI techniques have been used, including characterizing network segregation and integration (Rubinov and Sporns, 2010). Here, nodes (ROIs) and the connectivity parameter (known as an edge or link) must be defined. Connectivity is represented by an adjacency matrix (either voxel × voxel or ROI × ROI) that examines the relationships across all pairs of regions. Several FND studies have applied graph theory rsfMRI, using connectome measurements including weighted-degree (centrality), clustering coefficient, small worldness, and link-level metrics (Amiri et al., 2021; Diez et al., 2020; Ding et al., 2013, 2014). For interpretability, nodes that are highly connected to other brain areas (indexed via centrality) are termed “hubs”. A hybrid seed-based graph theory approach that allows for the characterization of information flow across brain networks, stepwise functional connectivity, has also been used in one study (Diez et al., 2019). Dynamic rsfMRI (e.g., sliding window approaches) characterize the intrinsic variance of network connectivity across the duration of the scan (rather than averaging BOLD signal for the entire scan); one FND study has used this methodology to date (Marapin et al., 2020). Lastly, while the above techniques extract network properties and subsequently perform statistical analyses, machine learning can identify features with potential diagnostic utility; a linear Support Vector Machine classifier was used in an FND cohort to identify network features with predictive diagnostic potential (Khosla et al., 2019; Wegryzak et al., 2019).

Additionally, while the full range of rsfMRI methodological considerations is beyond the scope of this article, head motion is a noteworthy issue given the presence of motor symptoms; as such, preprocessing and statistical analyses should explicitly address head motion artifacts (Power et al., 2015). Several studies have included head motion corrections.
7. Grey Matter Characterization

Given that brain structure–function relationships are closely coupled, a growing body of literature characterized grey matter in patients with FND. To date, these approaches include manual tracing (Atmaca et al., 2006, 2016), voxel-based morphometry (VBM) (Aybek et al., 2014a; Espay et al., 2018c; Kozlowska et al., 2017a; Labate et al., 2012; Maurer et al., 2012; Perez et al., 2017a, 2017b, 2018b; Riederer et al., 2017) and surface-based methods (Labate et al., 2012; Nicholson et al., 2014; Nigro et al., 2019; Osipova et al., 2019; Perez et al., 2018a; Ristic et al., 2015; Tomic et al., 2018; Vasta et al., 2018; Williams et al., 2018) (see Table 3). Manual tracing, historically considered the gold standard, requires a skilled neuropathologist to hand trace ROIs. This is a time- and resource-intensive process, with variable intra- and inter-operator reliability (Morey et al., 2009). However, manual tracing can provide a good solution to quantifying volumes in relatively discrete subcortical structures. In FND, studies have used manual tracing to quantify basal ganglia, thalamic and pituitary volumes (Atmaca et al., 2006, 2016).

In FND research, manual tracing has been largely replaced by automatic methods such as VBM and surface-based morphometry. VBM is a fully-automated process that statistically analyzes each T1 anatomical scan at the voxel-level. This procedure requires that images be transformed to a common anatomical space to assure correspondence across subjects, generally using the Statistical Parametric Mapping (SPM) (Ashburner and Friston, 2000) or FMRIB Software Library (FSL) VBM analysis pipelines (Jenkinson et al., 2012). Other important VBM steps include tissue segmentation, where normalized images are separated into grey matter, white matter and cerebrospinal fluid components using tissue probability masks, and smoothing, where data is made more compatible with the Gaussian field model. Some processing steps can introduce variability in statistical analyses, such as co-registration and partial-volume effect concerns (Larvie and Fischl, 2016). Several studies in FND have employed VBM to perform whole-brain (Aybek et al., 2014a; Espay et al., 2018c; Kozlowska et al., 2017a; Labate et al., 2012; Maurer et al., 2018; Perez et al., 2017b) or ROI-based analyses (Nicholson et al., 2014; Perez et al., 2017a, 2017b). Additionally, while surface-based analyses have been more widely used for cortical thickness measurements, one FND study combined VBM and voxel-based cortical thickness analyses (Aybek et al., 2014a).

Surface-based, semi-automated algorithms implemented in tools like FreeSurfer (http://surfer.nmr.mgh.harvard.edu/fswiki) enable the quantification of grey matter architecture based on reconstructed grey

### Table 2

<table>
<thead>
<tr>
<th>Technique</th>
<th>Methodological Description</th>
<th>Strengths &amp; Weaknesses</th>
<th>FND Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplitude of Low Frequency</td>
<td>Frequency-domain analyses based on power spectrum reflecting spontaneous regional neural activity.</td>
<td>ALFF has better reliability in grey matter than fALFF. ALFF is more sensitive to individual differences, while fALFF may be more prone to bias from physiological noise.</td>
<td>Li et al., 2015a; Maurer et al., 2016; Spagnolo et al., 2020</td>
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<tr>
<td>Fluctuations (ALFF)/Fractional</td>
<td></td>
<td>Readily interpretable. Findings are dependent in part on seed selection. Approaches to seed selection include using anatomical atlases, coordinates informed by the literature (e.g., meta-analysis) or task-based activations among other possibilities.</td>
<td>Allendorfer et al., 2019; Bank et al., 2017; Canu et al., 2020; Lee et al., 2018; Li et al., 2015a,b; Maurer et al., 2016; Morris et al., 2017; Spagnolo et al., 2020; Szafarski et al., 2018; van der Kruis et al., 2012</td>
</tr>
<tr>
<td>ALFF (fALFF)</td>
<td></td>
<td>Techniques are model free and not dependent on seed selection. In ICA, user pre-specifies or estimates the number of components. Once voxels are grouped together, the user discerns which data sets reflect neural organization and which reflect physiological noise.</td>
<td>van der Kruis et al., 2014; Monsa et al., 2018; Canu et al., 2020</td>
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<tr>
<td>Seed-Based rsfcMRI</td>
<td>Evaluates correlations between time series in a given seed (ROI) compared to other brain areas to identify spatially distinct networks.</td>
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<tr>
<td>Data-Driven Component and</td>
<td>Analyses aim to reduce the dimensionality of whole brain data into a smaller set of networks, using approaches such as independent component analyses (ICA) and clustering analysis.</td>
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<tr>
<td>Clustering Approaches</td>
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<tr>
<td>Graph Theory Network</td>
<td>Characterize the functional connectome using a correlation matrix and defining the nodes (ROIs) and connectivity strength measurements (links or edges).</td>
<td>Allows for the study of both of specific networks (segregation) as well as interactions across networks (integration). Techniques to define nodes include use of anatomical atlases and voxel-based approaches. Procedures are computationally demanding and multiple comparison considerations are important. Clinical translation of certain graph theory network properties to brain networks can be challenging.</td>
<td>Amiri et al., 2021; Diez et al., 2019, 2020, Ding et al., 2013, 2014</td>
</tr>
<tr>
<td>Applications</td>
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<tr>
<td>Dynamic (Sliding Window)</td>
<td>Characterizes the intrinsic variance of network connectivity across the duration of the resting-state scan (rather than averaging BOLD signal for the entire scan)</td>
<td>Allows for the quantification of fluctuations in resting-state connectivity across the duration of the scan. Window length selection is somewhat arbitrary and approach can be sensitive to outliers.</td>
<td>Marapin et al., 2020</td>
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<tr>
<td>rsfcMRI</td>
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<tr>
<td>Machine Learning</td>
<td>Analyses aim to distinguish a given patient group from comparison cohorts. Predictions can also be applied to characterizing relationships between non-imaging measures of interest and individual differences.</td>
<td>In unsupervised approaches, mathematical computations seek to disentangle explanatory variables in rich, unlabeled rsfcMRI data. Other approaches are supervised with greater user input regarding criteria for classification. For classifier-based analyses, the specificity and sensitivity of the findings can be calculated. Computations generally require large sample sizes and similar to graph theory, this approach is computationally demanding.</td>
<td>Wegrzyk et al., 2018</td>
</tr>
</tbody>
</table>

The Abbreviations: FND, functional neurological disorder; BOLD, blood-oxygen-level-dependent; rsfcMRI, resting-state functional connectivity magnetic resonance imaging; fMRI, functional magnetic resonance imaging; ROI, region of interest.
matter, white matter and pial surface boundaries (Fischl, 2012). Vertex measurements from these surfaces enable the calculation of several morphometric determinations, including cortical thickness, surface area, and curvature. Automated tracings using FreeSurfer also allow segmentation of subcortical structures, enabling volume measurements. While in vivo surface-based calculations have been validated against post-mortem measurements (Rosas et al., 2002), visual inspection of segmentation results is required to ensure that surface determinations were accurately demarcated. In the FND literature, whole-brain cortical surface analyses have been commonly employed (Labate et al., 2012; McSweeney et al., 2018; Negri et al., 2019; Ospina et al., 2019; Perez et al., 2018a; Ristic et al., 2015; Tomic et al., 2018; Vasta et al., 2018; Williams et al., 2018), with cortical thickness measures extracted in all studies. Additionally, one study combined surface-based measurements with a random forest machine-learning algorithm to investigate structural MRI characteristics that distinguished patients with FND-seiz compared to healthy controls (Vasta et al., 2018). Other multivariate analyses such as source-based morphometry have yet to be used in patients with FND.

Grey matter characterization using neuroimaging techniques also has general limitations that are important considerations, such as insufficient contrast in T1-weighted sequences to completely delineate deep grey matter structures (Pagonzzi et al., 2019). False positive rates may also be higher for volume and surface area calculations compared to cortical thickness measurements (Greve and Fischl, 2018).

8. White Matter Characterization

White matter characterization using diffusion-weighted imaging in FND is in its early stages. Diffusion-weighted imaging, commonly diffusion tensor imaging (DTI), quantifies the movement of water molecules along axons and allows for an in vivo characterization of a) the local microstructural white matter integrity, and b) the reconstruction of white matter connections using tractography (Jones et al., 2013). Tractography-based graph theory analyses may further elucidate structural brain networks (Rubinov and Sporns, 2010).

Voxel-based approaches to characterize white matter in patients with FND rely on scalars derived from DTI, most commonly fractional anisotropy (FA, a measure of microstructural integrity) and mean diffusivity (MD). These have been investigated in FND cohorts using tract-based spatial statistics (TBSS, i.e. voxel-wise analysis of skeletonized local diffusion measures) (Jungillijens et al., 2021; Lee et al., 2015; Sone et al., 2019; Tomic et al., 2018) and voxel-based analysis (Sojka et al., 2021). Alternatively, individual white matter tracts or a whole-brain connectome can be constructed using tractography. FND studies have applied both deterministic (Ding et al., 2013; Hernando et al., 2015; Li et al., 2015b) and probabilistic tractography (Diez et al., 2021; Jungillijens et al., 2021; Sojka et al., 2021). Furthermore, three of the aforementioned studies (Diez et al., 2021; Ding et al., 2013; Sone et al., 2019) used graph theoretical analysis to study the structural connectome of patients with FND. One study employed network lesion mapping to identify the grey matter origins of white matter findings (Sojka et al., 2021). In addition to conventional diffusion-weighted imaging, high angular resolution diffusion imaging (HARDI), which can more accurately delineate crossing fibers, has been applied in a FND-seiz cohort; this study also used the neurite orientation dispersion and density indices (NODDI) toolbox to characterize neurite dispersion, density and isotropic-free water volume fraction (Goodman et al., 2020). See Table 4 for a description of white matter approaches published in FND to date.

While diffusion-weighted imaging is a valuable tool, there are methodological considerations. Due to its reliance on detecting small displacements of water, DTI is susceptible to head motion artifacts (Yendiki et al., 2014). Furthermore, conventional DTI measures only one overall direction and degree of isotropy per voxel. In voxels containing crossing fibers, this leads to difficulties in estimating the underlying ‘true’ diffusion directions contributing to the overall signal. Approaches such as HARDI may more reliably account for crossing fibers, but acquisition times are longer than conventional DTI (Schilling et al., 2018).

9. Other Imaging Approaches

Besides functional/structural MRI and diffusion-weighted techniques, other neuroimaging modalities used in FND research include nuclear medicine (i.e., PET and SPECT), magnetic resonance spectroscopy (MRS) and near-infrared spectroscopy (NIRS). Nuclear medicine approaches have been applied to FND populations during both rest and task performance (Czarnecki et al., 2011; Galli et al., 2019; Marshall et al., 1997; Schrag et al., 2013; Song et al., 2014; Spence et al., 2000; Vuilleumier et al., 2001). Interictal and ictal SPECT, and interictal PET, have been used to differentiate FND-seiz and epileptic seizure cohorts (Baslet et al., 2021; Biraben et al., 1999; Neiman et al., 2009; Olver et al., 2019; Varma et al., 1996).

Table 3
Grey matter characterization approaches performed in functional neurological disorder to date.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Methodological Description</th>
<th>Strengths &amp; Weaknesses</th>
<th>FND Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manual tracing</td>
<td>Quantification of grey matter structures based on tracings by hand of the whole brain or regions-of-interest.</td>
<td>Historically considered the gold standard as it provides accurate identification of neural structures, and is particularly useful for small subcortical and limbic structures. Time- and resource-demanding process not readily applicable to large datasets.</td>
<td>Atmaca et al., 2006, 2016</td>
</tr>
<tr>
<td>Voxel-based morphometry (VBM)</td>
<td>Statistical comparison of grey-matter intensities for each voxel between participants.</td>
<td>Fully automated process that can be applied to large datasets to quantify voxel-level grey matter density. Several processing steps may be prone to variability, including co-registration and partial-volume effect concerns.</td>
<td>Aybek et al., 2014a; Espay et al., 2018c; Kozloska et al., 2017a; Labate et al., 2012; Maurer et al., 2018; Perez et al., 2017a,b, 2018b; Riederer et al., 2018</td>
</tr>
<tr>
<td>Surface-based morphometry</td>
<td>Reconstruction of the surfaces between grey matter, white matter and pial surface, allowing for the calculation of cortical metrics (thickness, surface area, curvature etc.)</td>
<td>Semi-automated process that can be applied to large datasets, with in vivo measurements correlating well with post-mortem measurements. Provides a volume-based segmentation stream for subcortical structures. Pial and white matter boundaries benefit from visual inspection and some metrics may be difficult to interpret (e.g., curvature).</td>
<td>Labate et al., 2012; McSweeney et al., 2018; Nicholson et al., 2014; Negri et al., 2019; Ospina et al., 2019; Perez et al., 2018a; Ristic et al., 2015; Tomic et al., 2018; Vasta et al., 2018; Williams et al., 2018</td>
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</tbody>
</table>

The Abbreviations: FND, functional neurological disorder.
In terms of neurochemical studies, two MRS studies have been performed in FND (Demartini et al., 2019; Simani et al., 2020). A NIRS study differentiated cardiogenic syncope from functional episodes during a tilt-test examination (Claffey et al., 2020), as it confirmed a stable cerebral tissue saturation index. While beyond the scope of this article, electrophysiological approaches including quantitative electroencephalography (EEG), spectral power, source localization, and event-related potentials have also been applied to FND (Barzegaran et al., 2015; Hallett, 2016a, 2016b; Kozlowska et al., 2017b, 2018; Meppelink et al., 2017; van der Salm et al., 2012). Promising techniques not yet used to study the pathophysiology of FND include ligand-based nuclear medicine techniques (including MRI-PET approaches), EEG-fMRI, and combined transcranial modulation–fMRI studies.

10. Individual Differences: Biomarkers of Symptom Severity, Illness Duration and Risk Factors for Developing FND

There is a small but growing number of studies reporting on the neural correlates of symptom severity and illness duration as well as variables that might influence risk of developing FND, particularly adverse childhood experiences. Across structural and functional neuroimaging studies, indices of patient-reported symptom severity (e.g., FND-seiz frequency, disability from functional dizziness) and/or illness duration have been used as covariates of interest in within-group designs in several FND cohorts (Atmaca et al., 2016; Aybek et al., 2014a; Diez et al., 2019, 2021; Jungiliggins et al., 2021; Kozlowska et al., 2017a; Labate et al., 2012; Lee et al., 2015; Li et al., 2015a,b; Maurer et al., 2018; McSweeney et al., 2018; Nicholson et al., 2014; Perez et al., 2017a; Riccelli et al., 2017).

Data on brain – risk factor relationships in FND are relatively sparse, and mostly inferred from trauma history. Notably, several studies have investigated brain – trauma burden relationships using self-report measures (e.g., Childhood Trauma Questionnaire, Life Events Checklist) in FND cohorts (Diez et al., 2020; Jungiliggins et al., 2021; Kozlowska et al., 2017a; Maurer et al., 2016, 2018; Perez et al., 2017a; Spagnolo et al., 2020). The role of other biopsychosocial-informed risk factors such as dissociation, alexithymia, personality profiles, insecure attachment, and social behaviors have received minimal attention to date (Labate et al., 2012; Ospina et al., 2019; Passamonti et al., 2018; Perez et al., 2018a; Sojka et al., 2019; van der Krujs et al., 2012, 2014; Williams et al., 2018). Furthermore, in some patients dissociation and alexithymia may represent intrinsic aspects of the same FND-related pathophysiology rather than risk factors per se. Sex-differences in the neurobiology of FND are also understudied (Maurer et al., 2018; Perez et al., 2017a; Williams et al., 2018).

Given the clinical heterogeneity found in FND, potential biological subtypes (e.g., intermediate phenotypes and endophenotypes) may be important considerations. For example, individual differences in childhood abuse burden correlated with corticobasal resting state functional connectivity in FND cohorts (Diez et al., 2020; Maurer et al., 2016); this suggests that a subset of individuals with FND (particularly those with adult onset) could potentially be conceptualized as having a delayed trauma-related disorder. Furthermore, differences in single-nucleotide polymorphisms of the tryptophan hydroxylase 2 gene in the context of studying gene by environment interactions have preliminarily identified biologically distinct FND subgroups (Spagnolo et al., 2020).

11. Imaging Predictive Biomarkers and Mechanisms of Treatment Response

With interest in developing FND treatments, recognized heterogeneity in outcomes among patients, and challenges in defining optimal clinical trial outcome measures (Nicholson et al., 2020; Pick et al., 2020), neuroimaging may help elucidate neural mechanisms and

<table>
<thead>
<tr>
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<th>Strengths &amp; Weaknesses</th>
<th>FND Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tract-based spatial statistics (TBSS)</td>
<td>Voxel-wise analysis of diffusion indices to quantify the local strengths of axonal directionality within white matter tracts.</td>
<td>Assesses white matter microstructural integrity, independent of local fiber orientation. Results are difficult to interpret in areas of crossing fibers, subject to partial volume effects in tracts and prone to head movement effects.</td>
<td>Jungilliggins et al., 2021; Lee et al., 2015; Sone et al., 2019; Tomic et al., 2018</td>
</tr>
<tr>
<td>Voxel-based analysis (VBA)</td>
<td>Voxel-wise approach to quantify diffusion indices throughout the subcortical white matter.</td>
<td>White matter assessments are not limited to a skeletonized map. If used in isolation, some difficulty relating findings to known fiber bundles. Accuracy of registration algorithms important.</td>
<td>Sojka et al., 2021</td>
</tr>
<tr>
<td>Tractography (deterministic)</td>
<td>Reconstruction of white matter connections based on a preset (deterministic) direction at each voxel.</td>
<td>More specific results than with probabilistic tractography, higher efficiency. Lower re-test reliability than probabilistic models, susceptible to noise, unable to account for inherent uncertainty in fiber orientation estimates.</td>
<td>Ding et al., 2015; Hernando et al., 2015; Li et al., 2015b</td>
</tr>
<tr>
<td>Tractography (probabilistic)</td>
<td>Reconstruction of white matter tracts based on a stochastic spatial distribution estimates of fiber orientation.</td>
<td>Shows greater reproducibility than deterministic models, and accounts for the inherent uncertainty in fiber orientation estimates. Less specific than deterministic models, with greater spatial dispersion of reconstructed streamline (may lead to more false-positive connections).</td>
<td>Diez et al., 2021; Jungiliggins et al., 2021; Sojka et al., 2021</td>
</tr>
<tr>
<td>Graph theory-based</td>
<td>Characterizes the structural connectome using nodal (cortical or subcortical regions-of-interest) and connectivity measurements (edges) derived from tractography.</td>
<td>Macroscopic representation of structural connectome, quantifying the relative structural connectivity between cortical regions. Results dependent on node segmentation, requiring assumptions to characterize white matter.</td>
<td>Diez et al., 2021; Ding et al., 2013; Sone et al., 2019</td>
</tr>
<tr>
<td>High angular resolution diffusion imaging (HARDI)</td>
<td>Measures diffusion signal along more gradient directions than conventional diffusion tensor imaging (DTI).</td>
<td>Can characterize both tensor metrics (e.g., fractional anisotropy) and tractography. Provides the orientation directions of multiple tracts found within a given voxel. Acquisition times are longer than traditional DTI sequences.</td>
<td>Goodman et al., 2020</td>
</tr>
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</table>

The Abbreviations: FND, functional neurological disorder.
predictors of treatment response. Notably, neural circuit profiles predicting treatment response may or may not overlap with pathophysiological mechanisms.

Three studies examined functional activations and/or connectivity changes following treatment interventions (Espay et al., 2019; Faul et al., 2020; Vuilleumier et al., 2001). In one study, 15 patients with functional tremor underwent 12-weeks of CBT, as well as pre- and post-treatment participation in three tasks obtained during a single fMRI scan session: finger tapping, basic emotion processing (affectively-valenced face viewing) and intense emotion processing (International Affective Picture System images) (Espay et al., 2019). Here, relationships between baseline depression scores and post vs. pre-treatment activation patterns were investigated. Another fMRI study performed in 14 patients with FND-movt measured baseline and post-treatment activations using an emotional Go/No-Go task in the context of participating in a 5-day multidisciplinary motor retraining treatment program (Faul et al., 2020). This study adjusted for depression and anxiety scores, and also investigated how baseline fMRI profiles related to treatment response. A SPECT study conducted in 7 patients with functional sensorimotor deficits probed blood flow patterns while symptomatic and 2–4 months later once symptoms resolved (treated with supportive psychotherapy and physiotherapy) (Vuilleumier et al., 2001). Additionally, two studies in 22 outpatients with mixed FND investigated how baseline resting-state functional connectivity (Dicz et al., 2019) and grey matter (Perez et al., 2018b) profiles related to prospectively collected 6-month clinical outcomes following individualized treatments (e.g., CBT, physical therapy): baseline depression and anxiety scores were used to contextualize findings. No prospective studies have evaluated relationships between white matter profiles and treatment outcomes. Real-time fMRI neurofeedback is another potential treatment to investigate in future FND research (Sukhodolsky et al., 2020).

12. Research Agenda

To summarize, FND neuroimaging research has grown over the past 20 years but continues to lag behind other prevalent neuropsychiatric disorders. Emerging themes include: i) brain dysfunctions in FND are distinct from feigning; ii) the pathophysiology of FND implicates multiple brain networks across functional and structural neuroimaging studies (e.g., abnormal salience/limbic-motor control and rTPJ-sensorimotor network interactions in fMRI studies); iii) linking neural mechanisms to etiological factors in FND is still in its early stages, as are biomarker studies of treatment response and prognosis. Task and resting-state fMRI have been the most widely used modalities to date, with few attempts to consider multimodal data. Replication has also been limited. Furthermore, while researchers have rapidly adopted “rule-in” examination signs and seminal features as core components of FND inclusion criteria, challenges remain regarding how (and if) to disentangle FND-related neural circuit profiles from other co-occurring mental and physical health symptoms. Likewise, questions regarding whether observed neuroimaging findings are disorder-specific, compensatory or linked to risk factors and confounds among other possibilities remain unanswered (Bégue et al., 2019).

To advance FND neuroimaging research over the next decade, we recommend the following research agenda. Note, this neuroimaging-focused research agenda is partially informed by recently published pathophysiology-focused research agenda formulations (Drane et al., 2020; Pick et al., 2019).

- **FND cohorts in neuroimaging studies require more detailed categorical and dimensional characterization across neurological, medical and psychiatric/psychological domains:** while emphasis on positive signs should remain a key inclusion criterion, future research should make concerted efforts to describe and quantify the range of functional neurological symptoms and other bodily symptoms experienced by individuals - most notably but not limited to pain, fatigue, and cognitive complaints (Maggio et al., 2020; Nicholson et al., 2020). Additional work is also needed to operationalize and further validate rule-in signs for FND. Regarding FND phenotypes, the majority of studies to date focused on a single subtype (e.g., FND-seiz), while others further narrowed inclusion criteria to specific phenotypes within overarching categories (e.g., functional dystonia). While these efforts remain important, many patients with FND have mixed symptoms. Thus, greater clarity regarding the range of functional neurological symptoms present is needed in research cohorts; ongoing efforts by the FND Society to better operationalize FND diagnostic criteria will also aid cohort characterization. Similarly, consensus self-report and other measurements assessing additional distressing bodily symptoms if widely adopted would advance this effort and aid future data aggregation (see below) (Pick et al., 2020). The presence of functional somatic disorders (e.g., fibromyalgia) and other neurological conditions (e.g., traumatic brain injury) should also be documented. Similarly, lifetime psychiatric symptomatology should be delineated using structured psychiatric interviews as well as dimensional psychopathological measurements; indices of adverse life experiences should be assessed across the lifespan. Medication use with known central nervous system effects should also be consistently detailed. Of note, caution should be taken to not overly emphasize the recruitment of “pure” forms of FND (those without neuropsychiatric comorbidities) – in part because the generalizability of such findings may be questionable. Furthermore, while we are recommending more robust cohort characterizations, we are not necessarily advocating for exhaustive assessments and recognize that investigators need to make tradeoffs depending on their study designs and research questions.

- **While healthy controls remain important, patient controls across neurological, psychiatric and medical diagnoses are needed to help delineate the specificity of observed FND findings.** Such patient controls could include individuals with chronic pain disorders, migraine, fibromyalgia, mild traumatic brain injury, major depression, generalized anxiety disorder, panic disorder, PTSD, dissociative disorders or mixed mood/anxiety psychiatric controls among other possibilities. Notably, some of these patient controls may have an overlapping pathophysiology with FND, observations that will nonetheless clarify disease mechanisms. Additionally, controls with similar symptoms driven by likely distinct neural mechanisms offer the opportunity to identify diagnostic biomarkers of FND; for example, comparing FND-seiz to epileptic seizures and comparing FND-movt to neurological disorders causing similar symptoms (e.g., primary dystonia). Lastly, it is beneficial to characterize healthy controls with the same measurements as those applied to patient groups.

- **Study designs should complement between-group approaches with relevant stratified between-group and within-group analyses.** Given that between-group (FND vs. controls) analyses are limited by the heterogeneity inherent to FND, stratified between-group analyses (FND-acute symptoms vs. FND-chronic symptoms; FND-prepubertal onset vs. FND-postpubertal onset) and within-group analyses provide important complementary information.

- **Longitudinal studies (naturalistic and pre/post treatment) are needed to investigate state vs. trait markers of FND pathophysiology, as well as to identify prognostic biomarkers and neural mechanisms of treatment response.** For example, while initial efforts have investigated structural and functional biomarkers of symptom severity cross-sectionally, it remains unclear how ‘responsive’ structural and functional brain profiles are to symptom fluctuations over time. Longitudinal studies may also help disentangle the importance of developmental trajectories and stress-diathesis model components in the pathophysiology of FND (Keynejad et al., 2019; Kozlowska, 2017).
• Within-group designs investigating neural circuit profiles associated with symptom severity and illness duration may be particularly informative in elucidating the pathophysiology of FND. As noted earlier, within-group designs can help account for co-occurring neuropsychiatric symptoms and improve statistical power; however, given that symptom severity and illness duration can also covary with other neuropsychiatric symptoms, secondary analyses should be performed with depression and anxiety scores (or similarly relevant factors) as covariates of noninterest.

• In addition to mapping symptoms and relevant psychological constructs (e.g., agency, attentional biases, expectation/infere-
ence, alexithymia, dissociation, interoception, conversion, catastrophizing etc.) to neural circuit profiles, relationships between brain areas and other biological markers (neuroendocrine (e.g., stress and sex hormones), autonomic (e.g., heart rate variability and electrodermal skin responses), neuro-inflammatory, genetic and epigenetic factors) should be inves-
tigated. Integrating a range of psychologically and biologicallyrelevant factors will help refine the understanding of links between what is observed in the brain and other bodily systems, as well as to overcome the dualism of separately studying psychological and neuropsychological factors. Such multi-level measurement efforts will also help mitigate potential interpretation biases. Nonetheless, it is also important to note that while complex psychological functions can undoubtedly be further understood through neuroscience (Kand-
el, 2013), neuroimaging techniques – in their present form – may not be able to fully delineate some relevant processes such as free will (Hallett, 2016). Furthermore, composite biomarkers that include a range of psychobiologically-relevant variables may potentially exhibit greater explanatory power than a single measurement.

• Multimodal (combined MRI, fMRI, DTI, EEG, and/or PET etc.) neuroimaging studies are encouraged to contextualize intrinsic structural and functional architectural profiles with task-related activation profiles. This is analogous to collecting data from a resting echocardiography and exercise stress test to obtain a more complete picture of cardiac functioning. In vivo neurochemical (MRS) and nuclear medicine-based neurotransmitter binding studies would likely also add insights into the pathophysiology of FND. Similarly, EEG-fMRI studies can provide improved temporal resolution, while fMRI-TMS studies can interrogate discrete brain circuits to provide causal information.

• Understanding how FND mechanisms and etiological factors relate to one another is important. This should include efforts to contextualize roles for potential sex differences, developmental tra-
jectories, stress coping, resilience, attachment, alexithymia and epigenetic / genetic factors. Contextualizing relationships across risk factors, including delineating moderators is another noteworthy research area.

• Larger sample sizes are needed to comprehensively perform secondary and post-hoc analyses aimed at adjusting for poten-
tial confounding variables, as well as to assist in ensuring that findings are robust and replicable. The future inclusion of test and replication cohorts in the same study would substantially increase the impact and clarity of neuroimaging findings.

• To robustly address many of the above considerations, FND researchers should establish large-scale international data sharing initiatives. One high impact consideration is to join the Enhancing NeuroImaging Genetics through Meta-Analysis (ENIGMA) Consortium, an international effort by leaders worldwide to bring together clinical neuroscience researchers to under-
stand pathological brain structure and function relationships, using MRI, DTI, fMRI & genetic data across patient populations (Thompson et al., 2020). ENIGMA’s ethos is that the best return on research investments will come from combining data to achieve large samples necessary to detect modest imaging/gene effect sizes that we now know are the rule rather than the exception for complex traits. A particularly relevant consideration that we are actively exploring is to have FND researchers establish the FND working sub-group as part of the ENIGMA-Dissociation Working Group (Reinders, 2020). Dissociative symptoms are common in FND and its frequently co-
occuring psychiatric conditions including PTSD, somatic symptom disorders, borderline personality disorder, anxiety disorders, and depersonalization/derealization disorder (Lyssenko et al., 2018). The ENIGMA-Dissociation Working Group is set up as an umbrella where individual subgroups investigate biomarkers of separate dis-
orders involving dissociative symptoms. The amalgamation of the within disorder specific dissociation biomarkers from these separate segments will ultimately inform the study of biomarkers for patho-
logical dissociation across disorders (Roydeva and Reinders, 2021). Additionally, disorder specific neural mechanisms could be investi-
gated by performing large-scale between-group comparisons across FND and its co-occurring psychiatric conditions. Participation in the ENIGMA Consortium would also allow access to neuroimaging data across a range of other relevant disorders (e.g. traumatic brain injury) (Dennis et al., 2020).

13. Conclusions

Neuroimaging research in FND has steadily matured alongside sub-
stantial progress made in the diagnosis and treatment of this population. This article provides readers with an overview of the state of neuroimaging research in FND, emphasizing conceptual and methodological considerations. A research agenda to promote high impact neuroimaging-focused research questions is also outlined. Given complexity inherent to the pathophysiology of FND, there is an urgent need for large-scale collaborations to more definitely answer key mechanistic questions regarding FND. Advancing the pathophysiology of FND, in part through multimodal neuroimaging approaches, has important ramifications not only for FND but also for a more holistic conceptualization of mental and physical health more broadly.

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Conflicts of Interest/Disclosures

D.L.P., honoraria from Harvard Medical School for continuing medical education lectures on FND and serves on the editorial board of Epilepsy & Behavior. A.A.A.P., Honoraria from Cobel Daruo,
RaymandRad and Tekaje; Royalty: Oxford University Press (Book royalties). A.J.C., paid associate editor of Journal of Neurology, Neurosurgery and Psychiatry (JNPP) and provides expert testimony in court on a range of neuropsychiatric topics including FND. A.J.E., grant support from the NIH and the Michael J Fox Foundation; personal compensation as a consultant/scientific advisory board member for Abbvie, Neuroderm, Neurocrine, Amneal, Acadira, Acorda, Kyowa Kirin, Sunovion, Lundbeck, and USWorldMed; honoraria from USWorldMed, Acadia, and Sunovion; publishing royalties from Lippincott Williams & Wilkins, Cambridge University Press, and Springer. M.H., inventor of patents held by NIH for an immunotoxin for the treatment of focal movement disorders and the H-coil for magnetic stimulation; in relation to the latter, received license fee payments from the NIH (from Brainway); on the Medical Advisory Boards of CALA Health and Brainways; research grants from Allergan for studies of methods to inject botulinum toxins, Medtronic, Inc. for a study of DBS for dystonia, and CALA Health for studies of a device to suppress tremor. W.C.L., has served on the editorial boards of Epilepsia, Epilepsy & Behavior, JNPP, and Journal of Neuropsychiatry and Clinical Neurosciences; receives editor’s royalties from the publication of Gates and Rowan’s Non-epileptic Seizures, 3rd ed (Cambridge University Press, 2010) and 4th ed (2018); receives author’s royalties for Taking Control of Your Seizures: Workbook and Therapist Guide (Oxford University Press, 2015); received research support from the US Department of Defense (W81XWH-17-0169), National Institutes of Health (NIH; NINDS SK23NS45902 [principal investigator]), PVAMC, Center for Neurorestoration and Neurorehabilitation, Rhode Island Hospital, the American Epilepsy Society (AES), the Epilepsy Foundation, Brown University, and the Siravo Foundation; on the Epilepsy Foundation New England Professional Advisory Board, the FND Society Board of Directors, and the American Neuropsychiatric Advisory Association Council; received honoraria for the AES Annual Meeting; served as a clinic development consultant at University of Colorado Denver, Cleveland Clinic, Spectrum Health, Emory University, and Oregon Health Sciences University; and provided medicolegal expert testimony. K.L., honoraria from the American Academy of Neurology and the International Parkinson and Movement Disorder Society, personal compensation for scientific advisory boards from Acorda. J.S., royalties from UpToDate and carries out expert witness work in relation to FND; runs a free website for people with FND, www.neurosymptoms.org. J.P. S., funded by the NIH, National Science Foundation, US Department of Defense (W81XWH-17-0169), State of Alabama, Shor Foundation for Epilepsy Research, UCB Pharma, Neuro Pace, Greenwich Biosciences, Biogen, Xenon Pharmaceuticals, Serina Therapeutics, and Eisai; served on consulting/advisory boards for Greenwich Biosciences, NeuroPace, Serina Therapeutics, LivaNova, UCB Pharma, Lundbeck, and Elite Medical Experts; and serves as an editorial board member for Epilepsy & Behavior, Journal of Epileptology (Associate Editor), Epilepsy & Behavior Reports (Associate Editor), Journal of Medical Science, Epilepsy Currents (Contributing Editor), and Folia Medica Copernicana.

References
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Janet, P., 1907. The major symptoms of hysteria; fifteen lectures given in the Medical School of Harvard University. Macmillan, New York.


