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Magnetic Resonance Imaging of Psychological Susceptibility and Resilience

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Given the complexity of most psychiatric disorders, they are notoriously difficult to study. Central to the challenge is that the pathophysiology is likely a distributed dysfunction across multiple different neural circuits (1). Thus, investigations into psychiatric diseases and their respective models must be able to scale from the level of molecules to whole-brain circuit analysis. Whereas there are numerous robust tools for studying the molecular and cellular mechanisms of psychiatric disorders, few research methodologies allow for systems-level analysis of the brain. Therefore, it is not surprising that the use of magnetic resonance imaging (MRI) has gained such widespread use and acceptance, especially in the context of human research. Interestingly, over the past 2 decades, MRI methodologies have been increasingly employed in studying animal models. While there are numerous benefits to using MRI as a tool for studying animal models, there are two major advantages. First, by conducting analogous MRI analysis between humans and animal models, there is an unparalleled chance to conduct directly translational research (2). Second, through the use of animal models it is possible to determine the molecular and cellular mechanisms and dynamics that manifest macroscopically as changes in MRI signal.

With respect to the first major advantage, Anacker and colleagues (3) used *ex vivo* MRI techniques to analyze neuroanatomic differences in mice undergoing chronic social defeat stress. Chronic social defeat stress has a longstanding history of serving as a training paradigm for creating depression-like and anxiety-like behaviors (4). Briefly, chronic social defeat stress involves social subordination of an intruder C57BL/6J male mouse by an aggressive CD-1 resident male mouse over multiple days. As a result of chronic social defeat stress, approximately two thirds of mice will display depression-like (4) and anxiety-like behaviors (susceptible), whereas another one third will develop only a mild anxiety-like phenotype (resilient). To understand differences in neuroanatomy between mice that were susceptible or resilient, Anacker *et al.* acquired and analyzed high-resolution structural and diffusion-weighted images of each phenotype.

Methodologically, to generate susceptible and resilient mice, C57BL/6J male adult mice were directly exposed to an aggressive CD-1 mouse for 5 minutes. After exposure, a perforated barrier separated the two mice for 24 hours, providing additional psychological

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stress. This sequence was repeated for 10 days with new aggressor mice. After the training session, the experimental mice were exposed to an open field arena without the aggressor mouse and then re-exposed to the arena with the aggressor mouse. For both exposures, measurements were made of the cumulative time spent in the interaction zone. The ratio of the time spent in the interaction zone in the presence of a naive CD-1 mouse to the time spent in the zone without the CD-1 mouse was used to determine whether the mice were susceptible (<1) or resilient (>1) to social defeat stress. In addition, the mice were assigned a *Z* score of social avoidance based on the time spent in the interaction zone, the time spent in the corners, and the ratio of time in the interaction zone with the aggressor present versus not present. Twenty-four hours later, the mice were perfused and decapitated for high-resolution structural imaging and diffusion-weighted imaging in the MRI scanner. From these imaging volumes, preselected brain regions of interest were compared with respect to their social avoidance score. Table 1 summarizes the results.

Notably, mice that were susceptible to chronic social defeat tended to exhibit a relatively larger ventral tegmental area, amygdala, hippocampus, and hypothalamus. Conversely, they had a relatively smaller cingulate cortex and nucleus accumbens. With respect to what these larger or smaller volumes represent, the results are consistent with previous neuronal morphology findings in the literature on chronic stress. Previous work has shown a trend toward smaller-volume spines in the nucleus accumbens (5), decreased dendrite and dendritic spine density in the cingulate cortex (6), and increased dendritic spine density and dendritic branching in the amygdala. Together, these findings agree with emerging evidence that changes in dendritic spine and dendrite morphology may partially explain the changes in volume observed at a macroscopic level with volumetric MRI techniques (7,8).

In addition to the volumetric analysis, Anacker *et al.* (3) used fractional anisotropy (FA) measures from diffusion tensor imaging in an analogous manner. With one exception (the hippocampus), correlations between the FA values and social avoidance scores were opposite those of the volumetric analysis. Because FA is a measure of the geometric constraint of diffusion along an axis, it often serves as a proxy for how neuronal components, classically axons in white matter, are aligned along an axis (9). However, when one is considering gray matter, the FA measure is less clear relative to the cellular basis. So why are the volumetric and FA correlations opposing? Speculatively, one can imagine that neuronal bodies with more branched dendrites with a higher density of spines will be less conducive to water diffusion along a particular direction (and have a larger volume) and thus be more isotropic. Therefore if a neuron has less dendritic branching and those dendrites have fewer spines, the gray matter will have a more concentrated diffusion axis (Figure 1). However, it is likely that there are also contributions from the glial cell density and structure, extracellular matrix, vascular structures, and myelination (10).

Looking beyond a region-of-interest analysis, MRI-based research also allows for analysis at a systems level. In the case of the work by Anacker *et al.* (3), they correlated volumetric measures of the different regions of interest with respect to the susceptible and resilient phenotype. Table 2 provides the correlative relationships with additional notes on where the susceptible animals clustered in the correlation. These results open up an opportunity to further explore dynamic relationships between these brain areas and their functional

consequences. For example, what are the mechanisms and functional significance of a larger ventral tegmental area and nucleus accumbens in susceptible mice?

Finally, in addition to testing *a priori* hypotheses, it is possible to test neuroanatomic differences across the whole brain with MRI analysis. Such an analysis provides a platform for the discovery of brain regions not previously associated with a particular model or illness. Using this approach, Anacker *et al.* (3) showed that social avoidance was negatively correlated with the thalamus, raphe nucleus, and bed nucleus of the stria terminalis. In addition, social avoidance was positively correlated with the habenula, periaqueductal gray, and cerebellum. Whereas these areas have well-known roles in other domains, their particular involvement in resilience versus susceptibility to social defeat stress is as of yet unclear.

In addition to exploring the role of the novel brain regions found in whole-brain analysis, future work should continue to build on the foundation laid by the work of Anacker *et al.* (3). In particular, most of the analyses were relative comparisons between susceptible and resilient groups. Such relative comparisons do not allow for an understanding of whether brain structures changed from baseline in the resilient and/or susceptible groups or whether the regions were different at baseline and predisposed the animals to their phenotypes. In a readdress, future work could include *in vivo* longitudinal imaging to determine the baseline neuroanatomy and any particular neuroanatomic changes with training, or any combination of the two. Alternatively, a direct comparison with a control group would establish whether the neuroanatomy of either phenotype was different from baseline. Finally, while highlighting the utility of magnetic resonance-based imaging, the study by Anacker *et al.* underscored the paucity of knowledge regarding the mechanistic underpinnings of volumetric and diffusion-weighted imaging. Preliminary work is available (7,8,10), but further rigorous experimentation using both functional and structural MRI, combined with detailed ultramicroscopic analysis, should focus on elucidating the mechanisms of changes in brain region volume, fractional anisotropy, and functional connectivity between structures before and after social defeat stress to obtain a detailed map of brain regions involved in stress and depression. Translationally, these findings have important implications for future research into the neurobiological underpinnings of stress and depression in terms of model validity. To that end, it will be important to frame the current findings in relation to human MRI studies of depression and ask how closely social defeat stress models the neuropathologic states observed in human depression., and whether we can then use this information to identify causal relationships between brain structures and circuits mediating depression-like behaviors in rodents. Presumably such studies might inform clinical investigation into novel targets for deep brain stimulation or novel therapies that restore volumetric alterations in vulnerable individuals.

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Figure 1.

Schematic representation of diffusion vectors with high-density vs. low-density dendritic branching. **(A)** In levels 1 and 2, apical dendrites show some branching. However, overall diffusion direction is along a single axis, as indicated by the arrow direction and magnitude (higher fractional anisotropy [FA]). In level 3, basal dendrites have relatively dense interweaving of dendrite branching; thus, water is equally likely to diffuse in all directions (equally restricted in all directions) and is relatively isotropic (lower FA). **(B)** Levels 1 and 2 show that apical dendrite density is mildly reduced, with higher FA compared with **(A)**. Level 3 has relatively less dense interweaving of basal dendrites and thus less restriction along the primary axis of diffusion, leading to increased overall FA relative to **(A)**.

Table 1

Volumetric and Fractional Anisotropy Region-of-Interest Analysis Comparing Susceptible and Resilient Mice

	Ventral Tegmental Area	Cingulate	Nucleus Accumbens	Amygdala	Hippocampus	Hypothalamus
Volume-Structural Magnetic Resonance Imaging	S > R	R > S	R > S	S > R	S > R	S > R
Social Avoidance Correlation	Positive	Negative	Negative	Positive	Positive	Positive
Fractional Anisotropy	R > S	S > R	S > R	S > R	S > R	S > R
Social Avoidance Correlation	Negative	Positive	Positive	Positive	Positive	Positive

R, resilient; S, susceptible.

Table 2

Correlational Analysis of Volumetric Data Among Regions of Interest With Respect to Susceptible and Resilient Mice

	NAcc	Cingulate	Hippocampus
VTA	Negative, ^a S—larger VTA, smaller NAcc	Negative, S—smaller cingulate, larger VTA	Positive, S—larger VTA, larger hippocampus
NAcc			Negative, ^a S—larger hippocampus, smaller NAcc
Cingulate			Negative, S—larger hippocampus, smaller cingulate
Hypothalamus			Positive, S—larger hippocampus, larger hypothalamus

NAcc, nucleus accumbens; S, susceptible; VTA, ventral tegmental area.

^aTrending significance.