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Research report

Revealing the cerebral regions and networks mediating vulnerability to depression: Oxidative metabolism mapping of rat brain

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HIGHLIGHTS

- Findings on neurobiology of depression in different animal models are hard to compare.
- We analyzed data on brain oxidative metabolism across five rat models of depression.
- Brain areas universally involved in stress response and vulnerability were revealed.
- Diathesis-stress approach suggests brain regions mediating adaptation to stress.
- Connectivity patterns in conditions of resilience and vulnerability are distinct.

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ABSTRACT

The large variety of available animal models has revealed much on the neurobiology of depression, but each model appears as specific to a significant extent, and distinction between stress response, pathogenesis of depression and underlying vulnerability is difficult to make. Evidence from epidemiological studies suggests that depression occurs in biologically predisposed subjects under impact of adverse life events. We applied the diathesis-stress concept to reveal brain regions and functional networks that mediate vulnerability to depression and response to chronic stress by collapsing data on cerebral long term neuronal activity as measured by cytochrome c oxidase histochemistry in distinct animal models. Rats were rendered vulnerable to depression either by partial serotonergic lesion or by maternal deprivation, or selected for a vulnerable phenotype (low positive affect, low novelty-related activity or high hedonic response). Environmental adversity was brought about by applying chronic variable stress or chronic social defeat. Several brain regions, most significantly median raphe, habenula, retrosplenial cortex and reticular thalamus, were universally implicated in long-term metabolic stress response, vulnerability to depression, or both. Vulnerability was associated with higher oxidative metabolism levels as compared to resilience to chronic stress. Chronic stress, in contrast, had three distinct patterns of effect on oxidative metabolism in vulnerable *vs.* resilient animals. In general, associations between regional activities in several brain circuits were strongest in vulnerable animals, and chronic stress disrupted this interrelatedness. These findings highlight networks that underlie resilience to stress, and the distinct response to stress that occurs in vulnerable subjects.

1. Introduction

Depression is a complex psychopathological condition that, due to its high prevalence and marked impact on individual and societal level [1] is a major focus area in neuroscience. The number of animal models of affective disorders is ever growing but the findings on the underlying neurobiology in these models are difficult to compare [2,3]. Genome-wide expression profiling studies with high level of methodological harmonization have revealed that similar depressive phenotypes elicited by different interventions associate with largely divergent patterns of gene expression [4–9], and that it may be difficult to separate depression-related and adaptive alterations [10,11]. This is not surprising if depression is brought about by non-adaptive alterations in the activity of coordinated whole

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brain networks [12] and the pathogenesis of depression may have a variety of seed regions from which the dysfunction will spread to further areas [13]. Alterations common to all depressive phenotypes should however be observable at the level of established dysfunctional neural networks.

Persistent neuronal activity is strongly dependent on and regulates the expression level of proteins involved in mitochondrial energy production. Cytochrome c oxidase is the rate-limiting enzyme of the mitochondrial respiratory chain and the activity of cytochrome oxidase is a valid index of the input signal to a brain area [14]. Cytochrome oxidase histochemistry offers the advantage of revealing relatively persistent activity levels. A number of animal models of affective disorders have been subjected to measurement of oxidative metabolism in many brain regions by cytochrome oxidase histochemistry [10,15–20]. These studies have identified many brain regions, primarily subcortical, as being associated with depression-like state with some consistency, and suggested that their activity may be strongly dependent on serotonergic tone [21]. Furthermore, functional connectivity as studied by inter-regional correlational analysis appears to be changed, *e.g.*, midbrain nuclei have lost connectivity with forebrain areas in depressed phenotypes [19]. Nevertheless, distinct animal models have again provided significantly divergent results. While one of the reasons may be the low statistical power in each study, it appears that the high model specificity, possibly related to the unique mechanisms behind producing the phenotype in each, clouds the core neurobiology common to the condition of being depressed.

Evidence from epidemiological studies strongly suggests that depression is caused by both genetic and environmental factors, and most potently by their combination [22]. It has been claimed that animal modeling of depression more often than not confuses depression with vulnerability to depression [23] by focusing on what rather is the increased ease to observe depressive phenotype and by missing the importance of stressful events to precipitate true depression. While distinction between vulnerability and depression may be difficult to maintain in animal models, the known etiopathogenesis of depression suggests that neurobiologies of depression vulnerability and stress response do need separation. This can be achieved by applying diathesis-stress approach across a number of different models of depression [24]. Different phenotypes of vulnerability to depression all by definition render the animal less adaptive to chronic stress.

Therefore, in the present analysis we combined data on regional oxidative metabolism from some previously published and some unpublished studies to address the following questions: (i) Which brain regions are involved in development of depressed phenotype across rat models? (ii) Which regional differences are characteristic to vulnerability phenotypes and which to response to chronic stress of different type? (iii) How does functional connectivity between brain regions reflect the state of vulnerability to depression and response to chronic stress? and (iv) Does the stress response in depression diathesis have a distinct pattern of regional oxidative metabolism?

2. Materials and methods

2.1. General procedure and animals

Data of five experiments with identical cytochrome oxidase activity measurement protocol were pooled. Animals were classified by two factors, pre-defined vulnerability phenotype and chronic stress, forming four groups: resilient/control ($n = 37$), vulnerable/control ($n = 33$), resilient/stress ($n = 27$) and vulnerable/stress ($n = 29$). Male rats were purchased from Scanbur BK AB (Sollentuna, Sweden) or Charles River Laboratories (Barcelona, Spain) at age of three weeks and experimental procedures were started when the animals were young adults, at the age of 2–3 months. All experiments were in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Included animal models

Five vulnerability model experiments, three of these including chronic stress paradigms, were represented in the dataset. (i) Chronic social defeat stress was applied to rats with persistently higher or lower level of expression of hedonic trait, as measured by sucrose preference [25]: rats with higher expression of hedonic trait had been found to be more sensitive to stress [10]. (ii) Chronic variable stress was applied to rats treated either with vehicle or with parachloroamphetamine (2 mg/kg) to elicit partial serotonergic denervation [15]. Previous studies had shown that after partial serotonergic denervation rats were more responsive to chronic stress [26], and this effect was mitigated by citalopram treatment [27]. (iii) Another chronic variable stress experiment was carried out in rats differentiated by positive emotionality: Rats with lower levels of emitting 50 kHz ultrasonic vocalisations during a stimulation resembling play-behavior [28] are more susceptible to chronic stress [16,29]. Groupwise comparison of cytochrome oxidase histochemistry data of these three experiments had previously been published [10,15,16], respectively. Two vulnerability models, with data on oxidative metabolism previously not published, were further added to analysis: (iv) rats with persistently lower levels of spontaneous exploratory activity that have anxious/depressive phenotype in a number of tests and many depression-related alterations in gene expression [4,30]. (v) Maternal separation of rat pups that causes a depressive-like phenotype in adulthood, as indicated by anhedonia and passive coping [31]. These five studies contributed data of, respectively, 34, 29, 33, 20 or 10 rats, with group sizes 5–12, but most often 7–9.

2.3. Cytochrome oxidase histochemistry and image analysis

In all experiments, the procedure was as previously described [15]. Briefly, sections were fixed for 5 min in 0.125% glutaraldehyde solution at 4 °C and washed. Next the sections were pre-incubated for 10 min with 0.0275% cobalt chloride and 0.5% dimethyl sulfoxide in 0.05 M Tris buffer with 10% sucrose adjusted to pH 7.4 with HCl. Thereafter the sections were washed and stained for 1 h at 37 °C in an incubation solution consisting of 0.05% DAB (3,3′-diaminobenzidine tetrahydrochloride, AppliChem), 0.0075% cytochrome c (Sigma, prepared using TCA), 5% sucrose, 0.002% catalase (Sigma) and 0.25% dimethyl sulfoxide. The reaction was stopped by introducing the slides for 30 min to 3.5% formalin and 10% sucrose in phosphate buffer. The sections were dehydrated in ethanol and cover-slipped. Regions of interest to be directly compared in data analysis were always stained in the same incubation medium. Stained and cover-slipped sections were digitized and saved. Images were analyzed with the Image J 1.34s freeware with the help of a rat brain atlas [32] by outlining every region of interest by hand and measuring across it a single background-corrected optical density value, derived by Rodbard calibration. Three independent measurements from individual tissue sections for each animal were averaged to produce a single data point.

2.4. Parachloroamphetamine administration

Parachloroamphetamine (PCA) was administered 9 days before the chronic variable stress regimen (CVS). PCA (Sigma) was dissolved in distilled water and administered in the dose of 2 mg/kg