1 **Title: Evaluation of molecular brain changes associated with environmental stress in**
2 **rodent models compared to human major depressive disorder: a proteomic systems**
3 **approach**

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9 **Short Title: Comparative preclinical systems evaluation of MDD**

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20

Abstract

Objectives: Rodent models of major depressive disorder (MDD) are indispensable when screening for novel treatments, but assessing their translational relevance with human brain pathology has proved difficult.

Methods: Using a novel systems approach, proteomics data obtained from post-mortem MDD anterior prefrontal cortex tissue (n =12) and matched controls (n = 23) were compared with equivalent data from three commonly used preclinical models exposed to environmental stressors (chronic mild stress, prenatal stress, social defeat). Functional pathophysiological features associated with depression-like behaviour were identified in these models through enrichment of protein-protein interaction networks. A cross-species comparison evaluated which model(s) represent human MDD pathology most closely.

Results: Seven functional domains associated with MDD and represented across at least two models such as "carbohydrate metabolism and cellular respiration" were identified. Through statistical evaluation using kernel-based machine learning techniques, the social defeat model was found to represent MDD brain changes most closely for four of the seven domains.

Conclusions: This is the first study to apply a method for directly evaluating the relevance of the molecular pathology of multiple animal models to human MDD on the functional level. The methodology and findings outlined here could help to overcome translational obstacles of preclinical psychiatric research.

Keywords

Major depressive disorder; systems biology; animal models; social defeat; proteomics

43 **1. Introduction**

44 Major depressive disorder (MDD) is the third leading cause of disability in the world and has
45 been projected to become the number one cause of disability by 2030 (World Health
46 Organization, 2004). However, current treatment options for this disorder remain limited with
47 only one third of patients reaching remission following initial therapy (Trivedi et al., 2006).
48 In order to develop novel and improved treatments, animal models of MDD are investigated
49 to evaluate the potential benefits of promising new drug candidates.

50 The aetiology of MDD is not well understood, the onset of the disorder is most likely
51 precipitated by a combination of genetic and environmental factors. Depression-related
52 behaviours have been reported in genetically modified rodent models (including
53 manipulations of the serotonin transporter (Slc6a4), corticotrophin-releasing factor (*Crh*) and
54 brain-derived neurotrophic factor (*Bdnf*)), following selective trait breeding, or after
55 pharmacological challenges (corticosterone and lipopolysaccharide administration) or
56 environmental stress exposure, as reviewed previously (Agid et al., 1999; Barkus, 2013;
57 Berton et al., 2012; Cryan and Holmes, 2005; Markou et al., 2009; McArthur and Borsini,
58 2006). Environmental stress is thought to be one of the most significant risk factors for the
59 development of MDD, especially in an interaction with genetic risk factors, early life stress
60 and ongoing stress may determine an individual's vulnerability to develop depression
61 (Charney and Manji 2004). Common environmental manipulations used in animal models of
62 MDD include social defeat (SD), social isolation, chronic mild stress (CMS), learned
63 helplessness, maternal deprivation and prenatal stress (PNS). However, these preclinical
64 models are thought to have varying degrees of construct, face and predictive validity (Nestler
65 and Hyman, 2010).

66 Although no rodent model reflects a given psychiatric disorder in its complexity, they may
67 represent certain neurocognitive or neurobehavioural endophenotypes, thus providing
68 insights into certain aspects of the underlying molecular pathology associated with different
69 symptom dimensions. Consequently, rodent models are most commonly used for preclinical
70 drug evaluations due to the advantages of a homogenous breeding background and the
71 potential for controlled experiments.

72 In this study, we have attempted to develop and apply a novel methodology of evaluating and
73 comparing the molecular changes associated with three widely used rodent models of MDD
74 (based on different environmental stressors, i.e. SD, CMS, PNS), to human MDD post-
75 mortem brains. Proteomic data obtained from rodent models and human disease was
76 evaluated using a network biology approach. Thus, it was possible to characterize and
77 compare functional patterns associated with the molecular changes between species and
78 models.

79 **2. Materials and Methods**

80 Detailed outlines of the statistical modelling, network filtering, GO term enrichment and
81 kernel techniques used in this analysis are described in a previous study (Cox et al., 2016).
82 Supplementary Figure 1 provides a step by step illustration of the methodology.

83

84 **2.1. Clinical and Preclinical Samples**

85 35 post-mortem anterior prefrontal cortex (Brodmann area 10 - BA10) brain samples were
86 obtained from the Stanley Medical Research Institute (Torrey et al., 2000). Samples were
87 derived from 12 MDD patients (with purely affective diagnoses and no psychotic features in
88 their disease course) and 23 healthy control (CT) subjects.

89 Tissue collection took place with the full informed consent of a first-degree relative to
90 comply with the Declaration of Helsinki. As outlined previously (Gottschalk et al., 2014),
91 there were no differences in grey and white matter volumes between samples, and no
92 significant differences in brain side, gender or secondary axis diagnosis of alcohol
93 abuse/dependency and drug abuse/dependency between patients and controls.

94 Three stress-based rodent models of MDD commonly used in preclinical research were
95 generated applying the standard protocols outlined below. Rats (*Rattus norvegicus*) were used
96 in the CMS and PNS models and mice (*Mus musculus*) were used in the SD model. At the
97 end of each protocol, rodents were sacrificed by decapitation, and frontal cortex tissue
98 samples were collected from both stressed samples and a matching number of unstressed
99 control samples. As in previous studies (Ma et al., 2009), the frontal cortex was defined as the
100 anterior portion of the cortex up to 2.15 mm rostral from bregma.

101 **SD**: 7-9 week old animals were exposed individually to 9-13 month old socially dominant
102 aggressor animals for ten minutes each day, for ten successive days, in a 1:1 social
103 dominance constellation as described previously (Berton et al., 2006; Krishnan et al., 2007).
104 The two mice were kept in the same cage separated by a Plexiglas screen for the remaining
105 24 hours for constant visual and olfactory cues, prolonging the defeat procedure. A different
106 aggressor mouse was used each day to minimize inter-aggressor variability. On day eleven,
107 all mice exposed to SD stress were subjected to the established social interaction test
108 (Krishnan et al., 2007) which employs a video-tracking system to score interaction/avoidance
109 behaviours towards an aggressor mouse. Following this test, all mice exposed to SD stress
110 were sorted into either susceptible or resilient groups, by comparing the interaction test score
111 ratio for each animal to established behavioural scores associated with defeat and resilience
112 (Berton et al., 2006; Krishnan et al., 2007). An interaction test score ratio of 1, in which SD
113 animals have displayed an identical amount of interaction with a social and a non-social

114 target in the presence of a formerly unknown mouse, has been previously defined as the
115 threshold for dividing defeated mice into susceptible and resilient groups. Twelve animals
116 deemed susceptible in accordance to the SD procedure (social interaction score <1) described
117 above, and 12 control animals which did not undergo defeat were sacrificed 24 hours after the
118 final social interaction testing and 48 hours after the last defeat (SD mice only).

119 **CMS**: 24 adult male rats were housed individually with free access to food and water. 12
120 animals were maintained as controls and 12 animals were exposed to a mild stress regimen
121 for three consecutive weeks, five days a week, based on a previously described CMS
122 procedure (Moreau et al., 1992; Willner et al., 1987). Stressors consisted of repeated periods
123 of confinement to small cages (24 × 10 x 9 cm), two periods of continuous overnight light
124 exposure, one 18 hour period of food and water deprivation followed by 2 hours of restricted
125 food access, one 18 hour period of water deprivation immediately followed by 1 hour's
126 exposure to an empty bottle, and one 18 hour period of group housing in a soiled cage. The
127 CMS protocol was evaluated through three commonly used behavioural tests. The sucrose
128 preference test (SPT) (Pothion et al., 2004) saw each animal given free access to 150ml of
129 sucrose solution and 150ml of water. The SPT tests for reduced responsiveness to positive
130 stimuli, which is symptomatic of depression in humans. The forced swim test (FST) (Herrera-
131 Ruiz et al., 2006) was then used to analyse whether animals showed inclinations towards
132 despair by placing them in a situation where they may perceive that escape from the water is
133 impossible (Borsini and Meli, 1988). Finally the tail suspension test (Pollak et al., 2010) was
134 used as an additional quantifier of behavioural despair. Animals from the stress group
135 displayed similar depression-related phenotypes in comparison to the control group as
136 expected from previous work (Yang et al., 2014). All animals were sacrificed via decapitation
137 following the end of the protocol.

138 **PNS**: Pregnant dams were obtained at gestation day 6-7 and randomly assigned to control or
139 prenatal stress groups after one week of acclimatisation. The stress groups were exposed to
140 varying stressors during their third trimester, from gestational day 14 until day 20, following
141 an established protocol (Sickmann et al., 2015). A different stressor was applied for each of
142 these days. Following birth, all dams and pups were housed in a single cage and left
143 undisturbed for 20 days. On postnatal day 21, pups were separated from their mothers and
144 kept single housed. In an evaluation of the PNS paradigm, young adult animals were
145 subjected to stress evaluation behavioural tests between postnatal days 50-70 (Sickmann et
146 al., 2015). Locomotor and rearing/climbing activity was assessed over a 24h period as
147 changes in circadian activity patterns can be symptomatic of depression-like behaviour. The
148 FST was used as in the CMS procedure. Similar to previous work (Sickmann et al., 2015),
149 animals from the stress groups displayed a phenotype related to increased behavioural despair
150 and decreased exploratory behaviour. Adult male offspring (10 from the control group and 10
151 from the stress group) were sacrificed at postnatal day 150.

152 **2.2. Label-Free LC-MS^E Analysis of Brain Tissue**

153 MDD tissue storage, preparation and proteomic abundance comparisons were performed as
154 previously defined (Gottschalk et al., 2014). Approximately 12-16mg of mouse tissue per
155 sample were used for the SD model and 22-28mg of rat tissue per sample were used for the
156 CMS and PNS models. A previously published protein tissue isolation protocol was applied
157 to all rodent model tissue samples (Ernst et al., 2012). Samples were added to a fractionation
158 buffer containing 7M urea, 2M thiourea, 4% CHAPS, 2% ASB14 and 70 mM DTT at a 5:1
159 (v/w) ratio (Martins-de-Souza et al., 2007). Sonification and vortexing (at 4°C for 30 min) of
160 the samples was carried out before centrifuging at 17,000g at 4°C. A Bradford assay (Bio-
161 Rad) was used to determine protein concentrations of the

162 supernatants in triplicate, using acetone to precipitate proteins (approximately 100 µg) from
163 each sample. 100 µL of ammonium bicarbonate (50 mM) was used to dissolve the
164 precipitates, before protein concentrations were determined. Reduction of protein sulfhydryl
165 groups was carried out using 40 µg of proteins and 5 mM DTT at 60 °C for 30 min.
166 Alkylation was carried out using 10 mM iodoacetamide and incubating in the dark at 37 °C
167 for 30 min. Protein digestion was performed using porcine tosyl phenylalanyl chloromethyl
168 ketone (TPCK)-treated trypsin at a 1:50 (w/v) ratio for 17 h at 37 °C. Reactions were stopped
169 via the addition of 8.8 M HCl at a 1:60 (w/w) ratio.

170 All tissue samples were analysed individually in technical duplicates using label-free liquid
171 chromatography-coupled tandem mass spectrometry (LC-MS^E). For the subsequent analysis,
172 the Swiss-Prot human reference proteome (Uniprot release, Sep 2014; 20 209 entries) was
173 used for peptide/protein identification. The protein sequences of the *Mus musculus* and *Rattus*
174 *norvegicus* UniProt reference proteome files were merged to create a joint database (retrieved
175 Sep 2014; total joint number of entries 24 577). LC-MS^E raw data were processed using the
176 ProteinLynx Global Server v.2.5. (Waters Corporation), and Rosetta Elucidator v.3.3 (Rosetta
177 Biosoftware), applying settings and procedures as reported previously (Gottschalk et al.,
178 2014). Following the export of all peptide signal intensities for each sample, pre-processing
179 and statistical analysis was performed in R (R Development Core Team, 2013). Non-unique
180 peptides were excluded. To avoid unspecific trypsin cleavage, only peptides with an amino
181 acid sequence ending in R or K were considered. The first two principal components were
182 used to identify sample outliers (Beniger et al., 1980) resulting in the removal of two CT
183 samples and one MDD sample, one stress sample from the PNS model and one stress sample
184 from the SD model. Log_2 transformation was applied to stabilize data variance.

185 Protein abundance changes for the human and rodent model comparisons (MDD compared to
186 CT, or stress versus non-stressed controls in the rodents, respectively) were determined using

187 a fixed effects linear model. This model adjusted for covariates in the post-mortem brain
188 samples regarding age, gender, diagnoses of alcohol or substance abuse, brain pH, brain side
189 and post-mortem interval (PMI). For the human tissue, the false discovery rate (FDR) was
190 controlled by adjusting *p*-values according to the Benjamini Hochberg procedure (Benjamini
191 and Hochberg, 1995) with a cut-off of 0.05. For each rodent model, the small sample sizes
192 were accounted for by determining the real *p*-values for each protein through a permutation
193 testing procedure as described previously (Cox et al., 2016).

194 **2.3. Protein-Protein Interaction Networks**

195 Protein-protein interaction (PPI) networks were created for MDD and the SD, CMS, and PNS
196 rodent models using the software package Cytoscape v3.2.1 (Cline et al., 2007), enabling the
197 comparison of MDD and rodent tissue on a functional level, based on the annotation of Gene
198 Ontology (GO) terms to the detected significant protein abundance changes described above.
199 The databases MINT (Zanzoni et al., 2002), IntAct (Hermjakob et al., 2004) and UniProt
200 (Apweiler et al., 2004) were used to retrieve all available known PPI between the significant
201 (corrected *p*-value $\leq$ 0.05) protein abundance changes and their respective first-order protein
202 interactors. Network nodes were filtered by taxonomy identifiers (9606 for *Homo sapiens* in
203 the MDD network along with 10116 and 10090 for *Rattus norvegicus* and *Mus musculus*
204 respectively in the rodent networks) while edges were filtered so that all connections other
205 than direct interactions or physical associations between proteins were excluded. The
206 structures of each network were characterized using three properties of complex networks:
207 average degree which reflects connectivity, characteristic path length and density.

208 **2.3.1. GO term enrichment**

209 The ClueGO (Bindea et al., 2009) Cytoscape package was used to compute GO term
210 enrichment on each PPI network. Settings and filtering methods were applied as described

211 previously (Cox et al., 2016). The statistical significance of each GO term was computed
212 using a two-sided hypergeometric distribution, determining whether GO terms occur at a
213 frequency greater than would be expected by each term. The significance of each term was
214 adjusted for FDR using the Benjamini Hochberg correction with a cut-off of 0.05.

215 **2.3.2. Kappa Score Grouping**

216 Functional grouping was applied to the list of terms for each network using the kappa score
217 (Huang et al., 2007), a metric which reflects the degree of the relationship between GO terms,
218 based on shared underlying proteins. A kappa score of 0.5 was used, as this requires a
219 relatively large number of shared proteins in order for terms to be grouped (Bindea et al.,
220 2009; Merico et al., 2010), ensuring that each group has a distinct biological functionality.
221 SD, CMS or PNS functional groups which did not contain at least two GO terms were
222 excluded from the analysis. A more stringent threshold was applied to the MDD functional
223 groups, as the MDD PPI network was a factor of ten larger than the rodent networks. This
224 size discrepancy derives from the information bias in GO annotations and protein databases
225 between human and rodent proteins. MDD functional groups which did not contain at least
226 ten GO terms were excluded from the analysis. Groups were named according to the most
227 significant GO term following the Benjamini Hochberg FDR correction mentioned in 2.3.1.

228 **2.3.3 Local Linear Embedding Kernel Group Augmentation**

229 A diffusion-type manifold embedding technique called a Local Linear Embedding (LLE)
230 kernel was applied to enhance the biological interpretation and comparability of each
231 functional group by augmenting them with closely related GO terms as previously outlined
232 (Cox et al., 2016). Manifold embedding techniques classify data points in particular
233 categories and are commonly used in bioinformatics. The kernel we apply here computes
234 similarity scores based on a geometric interpretation of manifold embedding interpreting

235 every GO term as a point on a virtual two-dimensional GO tree. The LLE kernel was selected
236 because it emphasizes short-range interactions between GO terms (Roweis and Saul, 2000)
237 and thus is typically used to group related proteins or genes (Lerman and Shakhnovich, 2007;
238 Li et al., 2010; Zare et al., 2011). Every given GO term in a functional group was analysed
239 for positive similarity values to other GO terms due to relative closeness on the GO tree. The
240 LLE kernel group augmentation is based on the concept that the functional annotations added
241 by this approach are highly likely to be related to biological functions already conveyed by
242 that group (and closely related to the underlying PPIs). Therefore, this procedure results in
243 more informative function groups of highly interconnected GO terms.

244 **2.4. Functional Comparison between MDD and Rodent Models**

245 Following augmentation, the percentage overlap of GO terms was computed between MDD
246 functional groups and those of the three rodent models, using a Z score transformation.
247 Related clusters of functional groups between MDD and each model were identified using
248 hierarchical clustering with the Ward's criterion metric. This enabled different functional
249 domains of the disease which are represented across all three models, to be determined,
250 where each domain consists of a vector of GO terms.

251 **2.4.1 Identification of Unique Functional Domains**

252 These functional domains were displayed by projecting the augmented MDD and rodent
253 model groups into Cytoscape, creating four separate graphs of GO terms, in which nodes
254 represent terms and edges connect the terms found to be related by the LLE kernel (see
255 "Local Linear Embedding Kernel Group Augmentation"). This projection enabled the
256 identification of a subset of GO terms behind each domain which are completely unique to
257 that domain, thus defining the biological functionality more precisely.

258 **2.4.2 Domain Comparison Using GO Term Similarity**

259 For each unique functional domain of human MDD represented across the three rodent
260 models, a numerical quantification was obtained for which model represented the human
261 domain most closely, by adapting an existing approach used in genetic research (Fröhlich et
262 al., 2006; Speer et al., 2005). A similarity score was computed between rodent and human
263 domains by first evaluating the pairwise similarity scores between individual GO terms in the
264 domain vectors using the LLE kernel, and then obtaining the average of the best matching
265 GO term similarity between the domains. The rodent and human domains are more similar,
266 the closer the similarity scores are to 1.

267 **<u>3. Results</u>**

268 **3.1. Protein Abundance Changes for Brain Tissue Comparisons**

269 1280 unique quantifiable proteins were identified across all post-mortem brain samples. 875
270 were identified across all SD samples, 749 across all CMS samples and 887 across all PNS
271 samples. Applying linear modelling followed by false discovery correction, found that 109
272 proteins were differentially expressed in MDD patients compared to CT individuals. Linear
273 modelling of the rodent models followed by permutation testing found 68 proteins to be
274 differentially expressed in stressed compared to CT mice in the SD model, 43 in the CMS
275 model and 30 in the PNS model. (Supplementary Tables 1-4 display these proteins and their
276 fold changes).

277 **3.2. PPI Networks and GO Term Enrichment Analysis**

278 By retrieving all available interactions from the protein databases UniProt, MINT and IntAct,
279 PPI networks were created for MDD and all three rodent models from the significant proteins
280 identified above and their respective first-order interactors. In order to characterize each of

281 these networks, several commonly used structural properties of complex networks were
282 computed, average degree, characteristic path length and density (Table 1).

283 The CMS and PNS networks displayed a slightly greater connectivity and density, potentially
284 because they are more compact as indicated by their smaller characteristic path length.
285 Following functional enrichment analysis of the networks, and Kappa score grouping of the
286 resulting terms, 77 MDD functional groups, 52 CMS groups, 41 SD groups and 9 PNS
287 groups were obtained. Each group corresponds to a specific biological process. Table 2
288 displays the top 5 groups for MDD and each rodent model, in order of significance.

289 3.3. Identification of Corresponding Functional Domains Between MDD and Rodent 290 Models

291 Kernel techniques were used to enhance the identified functional groups, as described in the
292 methods section. Subsequently the percentage overlap was computed between MDD and
293 rodent model groups. It was found that groups which clustered together and overlapped are
294 involved in closely related biological processes, resulting in the identification of seven
295 functional domains of MDD in the post-mortem brains – “transport, localization and cellular
296 import/export”, “development/differentiation and immune system”, “cytoskeleton and
297 DNA/RNA processes”, “carbohydrate metabolism and cellular respiration”, “nucleic acid
298 metabolism and ATP/GTPase activity”, “intracellular signalling/regulation & post-
299 translational modification” and “cellular response and receptor signalling”, which are also
300 represented by the GO terms based on protein changes detected in the rodent models. The
301 CMS model represented all seven of these domains and the SD and PNS models represented
302 five, respectively. The domains are shown in Figure 1. Prior to quantifying which model was
303 most representative of MDD for each functional domain, the individual groups of GO terms
304 behind the seven domains for MDD and all three models were projected into Cytoscape. The

305 resulting networks of GO terms (Figure 2) enabled the identification of a vector of terms
306 which are unique to a certain domain, for both MDD and each of the three preclinical models.

307 3.4. Quantification of Most Representative Rodent Model via GO Term Similarity 308 Methods

309 The vectors of GO terms for each domain were compared by computing scores based on GO
310 term similarity, enabling the quantification of the model that represented MDD most closely
311 (Table 3).

312 The scores indicated that overall the SD model represented MDD most closely (to a greater
313 extent than the other two models) for the functional domains "transport, localization &
314 cellular import/export", "cytoskeleton & DNA/RNA processes", "intracellular
315 signalling/regulation & post-translational modification" and "cellular response & receptor
316 signalling". The SD and CMS models were particularly close to MDD for three functional
317 domains where the similarity scores between models and the disease are greater than 0.5 -
318 "transport, localization & cellular import/export", "development/differentiation & immune
319 system" and "cellular response & receptor signalling."

320 4. Discussion

321 Despite the high prevalence and considerable clinical impact of MDD, progress in
322 understanding its pathophysiology has proved difficult, thought to be due to the
323 heterogeneous and complex nature of the disorder (Krishnan and Nestler, 2008). Animal
324 models of MDD have been utilised for the identification of novel drug targets and have
325 provided new insights into the genetic and molecular alterations which are thought to
326 underpin the disorder (McArthur and Borsini, 2006). However, challenges have arisen in
327 comparing alterations in animal models with the underlying molecular disease factors in

328 MDD measured in serum (Chan et al., 2014) or brain tissue (Gottschalk et al., 2014).
329 Therefore, most comparisons are based on behavioural phenotypes, sometimes additionally
330 supplemented with histological and electrophysiological data. This has led to difficulties in
331 bias and irreproducibility, as many of the behavioural characteristics for MDD cannot be
332 translationally evaluated in animals (Krishnan and Nestler, 2011).

333 Here, we present a novel systems biology approach allowing the comparison of brain changes
334 from three rodent models with those observed in human MDD brains. Thus, we were able to
335 assess which model reflects changes in MDD most closely for various pathophysiological
336 features of the disease. The approach seeks to compare human and animal brain tissue on a
337 functional level using GO terms annotated to proteins. Proteomic analysis has several
338 advantages over genomic/transcriptomic analysis, as protein changes represent the
339 transcribed and translated genetic information resulting from epigenetic and mRNA
340 modifications more closely reflect the disease pathophysiology (Bayés and Grant, 2009).
341 Twin studies, for example, have approximated the heritability of MDD at 37% suggesting
342 that genetic investigations may not be the optimum basis for a comparison (Sullivan et al.,
343 2000). Similar to the case of schizophrenia and other psychiatric disorders, evidence suggests
344 that MDD is a polygenic disorder with the disease phenotypes arising as a consequence of
345 many small risk genes together with environmental factors (Lohoff, 2010).

346 Through this approach, we were able to quantify seven functional domains identified as
347 altered in MDD post-mortem brain tissue, which are all represented across two or more of the
348 investigated animal models. We showed that the four functional domains "transport,
349 localization & cellular import/export", "cytoskeleton & DNA/RNA processes", "intracellular
350 sig./regulation & post-translational modification", and "cellular response & receptor
351 signalling", are represented most closely by the SD model. Previous rodent studies of SD
352 stress have found brain changes implying impaired cell proliferation mechanisms (Schmidt

353 and Duman, 2007) which is consistent with our finding that the SD model is characterised
354 especially through intracellular signalling and post-translational modification. In addition, we
355 were able to demonstrate that the functional domains “development/differentiation & immune
356 system” and “carbohydrate metabolism & cellular respiration” are represented most closely
357 by the CMS model. It is unsurprising that immune dysfunction was represented strongly in
358 this model as disturbed HPA function, dampened neurogenesis and increased oxidative stress
359 have been reported in rat CMS models (Farooq et al., 2012; Van Bokhoven et al., 2011). The
360 “development/differentiation & immune system” domain is also strongly represented in the
361 SD model. This is in line with previous studies demonstrating that SD stress induces pro-
362 inflammatory signalling including increased monocyte trafficking via a variety of immune-
363 regulatory pathways found to be dysfunctional in the CNS of MDD patients (Christoffel et al.
364 2011; Monje et al. 2011). It is interesting to note that the PNS model displayed low similarity
365 scores for four of the five functional domains of MDD. The PNS model also yielded the
366 fewest protein abundance changes between stressed and non-stressed states and we cannot
367 exclude the possibility that this reflects the wide range of adaptations in rodents exposed to
368 prenatal stress. Although most experiments show a clear effect of PNS on the phenotype,
369 substantial variation can be observed across both pregnant dams and pups within a study
370 (Boersma and Tamashiro, 2015). On the other hand animals analysed in the SD model were
371 selected for susceptibility to social stress, potentially increasing intra group homogeneity.

372 This study supports the notion that systemic commonalities exist between MDD brain tissue
373 and rodent models of the disease. This is the first proteomic approach for a cross-species
374 comparison which combines non-hypothesis driven label-free LC-MS^E profiling of matched
375 post-mortem brain samples with a novel functional analysis procedure. There are multiple
376 strengths of the methodology. At the protein annotation stage, a joint rodent database was
377 used to avoid bias towards a particular species. Protein changes were identified using a

378 statistical approach which accounted for the variance present in the data due to common
379 confounders such as age, sex and substance abuse, while simultaneously avoiding over-
380 fitting. Preclinical studies typically explore the functional meaning of the changes seen in sets
381 of individual proteins through pathway analyses, while this study sought to examine
382 functional similarities between species through enrichment of protein-protein interaction
383 networks. These networks account for the complex way in which proteins influence each
384 other at multiple levels, and how their interactions underpin cellular processes on a systems
385 level, which cannot be represented by pathway analysis alone, thus expanding the functional
386 scope of the study. Directionality in terms of protein signalling is not accounted for in this
387 study, but as yet, there is only limited annotation information available allowing to account
388 for directionality in PPI networks in an unbiased manner.

389 The method outlined in this paper is intended as a template which could be used for future
390 studies comparing further animal models of MDD to the human disease, such as genetic
391 mouse models. Despite taking steps to reduce a rodent species-specific bias through a joint
392 protein database, we are aware that full comparability of preclinical models might only be
393 achievable on a species specific level. As both mice and rats come with possible advantages
394 to potential future experimental designs (tissue amount and sample size, available
395 behavioural paradigms, ease of genetic manipulation) our results regarding the comparative
396 validity have to be interpreted with caution. In addition, it should be noted that the applied
397 proteome extraction protocol and the subsequent in silico analyses in both human post
398 mortem brain and rodent tissue can only deliver an approximate view of the underlying
399 proteomes as multiple cell types are used simultaneously and are therefore represented jointly
400 in the significant abundance changes. Subsequent analyses should consider the fractionization
401 of samples, rendering the analyses of targeted sub-proteomes possible, e.g. the extraction of
402 integral membrane proteins.

In summary, we have shown that different environmental stress models of MDD can represent individual functional aspects of the disease more closely than others. The development of a methodology which can quantify molecular similarity between preclinical models and MDD in this way is needed, as it is generally agreed that individual models are unlikely to mirror the full extent of the human disease (Czéh et al., 2016). Therefore, a combination of models, each mirroring different molecular changes found in the human pathophysiology may be the way forward in preclinical research. Theories that MDD could be a constellation of diseases, manifesting in behavioural symptoms which correlate with different neurobiological adaptations (Carboni, 2013), suggest that preclinical models targeting different symptom clusters are likely to be of increasing importance in the search for novel pharmacological compounds. There is a pressing need for pharmacological interventions that differ from current approaches which have largely focused on monoaminergic neurotransmission (Brigitta, 2002). Future research should aim to re-evaluate these disease-model comparisons incorporating proteomic analysis from genetic and pharmacological models. The ability to apply this novel method to conduct a direct functional comparison between multiple preclinical models and MDD will help gain greater insights into the underlying molecular and cellular mechanisms behind behavioural abnormalities and their response to pharmacological interventions, as we attempt to obtain a greater understanding of the consequences of environmental stressors in the context of affective disorders.

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The funding agencies had no role in designing the study, the data collection, the data analysis and the preparation of the manuscript.

Conflict of Interest

The authors declare the following competing financial interest(s): SB is a director of Psynova Neurotech Ltd and PsyOmics Ltd. The other authors declare no conflict of interest.

Figure Captions

Figure 1. Identification of functional domains of human MDD represented across two or more rodent models. Vertical axes on the right side represent rodent functional groups. Hierarchical clustering using Ward's criterion was used on both vertical and horizontal axes to identify related clusters of groups for both MDD and each model.

(A Prenatal Stress

(B) Chronic Mild Stress

(C) Social Defeat

Figure 2. Networks of GO terms representing the functional domains of MDD which were identified across at least two rodent models. Projection of significantly overlapping GO terms into functional networks enabled the identification of a vector of unique terms underlying a particular domain for both MDD and each rodent model. Representative GO terms for each domain are highlighted.

(A) MDD (B) Prenatal Stress (C) Chronic Mild Stress (D) Social Defeat

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