"Domain Gauges": A Reference System for Multivariate Profiling of Brain fMRI Activation Patterns Induced by Psychoactive Drugs in Rats

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PURPOSE: Pharmacological magnetic resonance imaging (phMRI) of the brain has become a widely used tool in **drug research**.[1] However, it would be helpful to condense the complex drug-induced brain-activation patterns into semantically meaningful metrics that can aid **informed decision making**. We present here a set of **multivariate metrics** termed "domain gauges", which are calibrated based on different classes of **reference drugs**. Each class represents a "domain" of interest, e.g., a therapeutic indication or a mode of action (MoA), and is characterized by the **unique activation pattern** it evokes in the brain. The domain gauges help positioning any observed activation pattern within the framework of reference drugs.

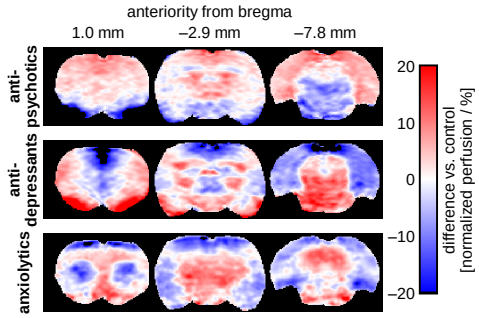

Figure 1: Mean coronal **perfusion maps** at selected anteriorities from bregma, obtained by pooling across all reference drugs of each of the 3 domains.

METHODS: We capitalized on our in-house **perfusion phMRI** database acquired over > 5 years from ~1000 rats subjected to **32 marketed and experimental drugs** and 2 non-drug treatments, each of the compounds administered at 1–5 doses, in 6–8 animals per dose. During imaging, animals were kept under isoflurane anesthesia. Continuous arterial spin labeling[2] MRI was performed on a 4.7 T / 40 cm Bruker Biospec. Coronal structural and perfusion images were acquired at 8 different anteriorities, and spatially normalized with SPM5 to an atlas with > 50 regions of interest (ROIs). To account for possible global brain perfusion changes, and to reduce inter-subject variability, individual perfusion maps were normalized slice-wise to the brain-mean value (set to 100 %). Mean perfusion per ROI was expressed as difference vs. negative control (vehicle). We defined 3 domains, with **(A) 7 antipsychotics**, **(B) 8 antidepressants** and **(C) 6 anxiolytics** as reference drugs, respectively, as well as 4 psychotomimetics/-stimulants, 2 non-drug treatments, 3 further anxiolytics and 4 anxiogenics as validation treatments. The construction of a domain gauge involved a principal component linear discriminant analysis (**PCA + LDA**) performed on the reference compounds of the respective domain. The "condensed" discriminant vector (i.e., the discriminant weights of those ROIs passing a loading-based criterion) was termed the **"domain profile"**. The application of a domain gauge to a given treatment yielded a **"classifier"** (score on the discriminant axis) as a measure of the overall strength of the response pattern in the "therapeutic" or "anti-therapeutic" direction, and a **"differentiator"** (angle between response pattern and discriminant axis in feature space) as a measure of scale-invariant deviation from the domain profile, both metrics along with confidence limits.

RESULTS: Mean **spatial maps (Fig. 1)** of perfusion changes induced by the reference drugs were highly specific for the respective domain. **Gauge construction:** In domain A, antipsychotics and psychotomimetics/-stimulants were already largely separated after the unsupervised PCA step. Similarly, in domain B, most of the antidepressants, especially the serotonergic compounds, were clustered on one side of the origin. This was also true for the benzodiazepines in domain C, which otherwise provided a more diverse picture. After the LDA step, the **domain profiles (Fig. 2)** revealed highly domain-specific patterns of altered perfusion across ROIs. **Gauge application (Fig. 3):** Gauge A showed positive classifiers for all antipsychotics except aripiprazole, negative classifiers for all psychotomimetics/-stimulants, and differentiators indicating larger deviations from the (inverse) domain profile for the latter than the former. On gauge B, only the serotonergic, but not the noradrenergic antidepressants showed positive classifiers under chronic administration, whereas the opposite was observed under acute administration. Chronic mild stress (CMS) and electroconvulsive stimulation (ECS) as non-drug treatments were classified in accordance with behavioral studies. Gauge C displayed positive classifiers of benzodiazepine agonists and one other anxiolytic, but more inconclusive responses for the other treatments, with differentiators suggesting substantial off-domain effects. Generally, domains A, B and C were dominated by 1st and 2nd generation antipsychotics, serotonergic antidepressants, and benzodiazepines, respectively.

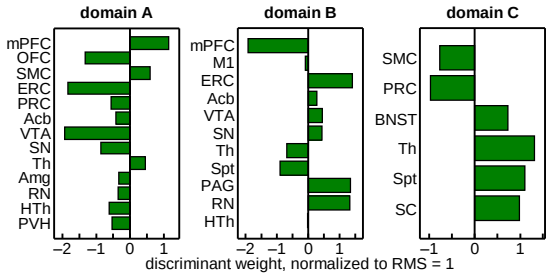

Figure 2: Domain profiles as identified from the ROI-based analysis. Bars show discriminant weights for those ROIs passing a loading-based criterion.

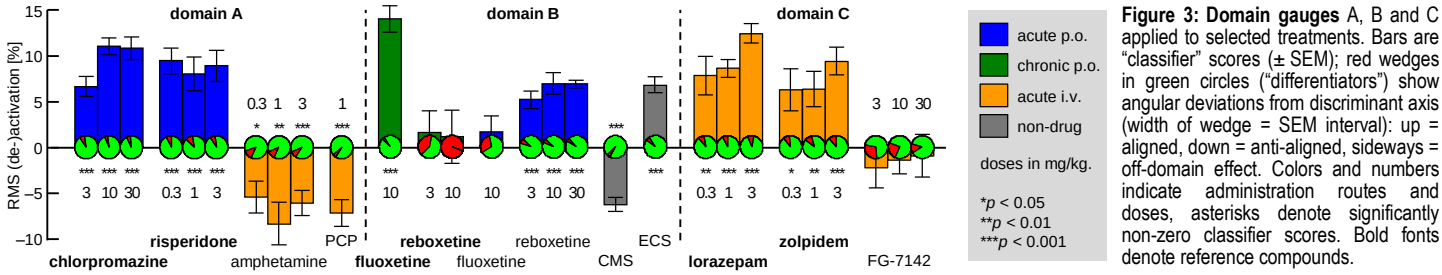

Figure 3: Domain gauges A, B and C applied to selected treatments. Bars are "classifier" scores (± SEM); red wedges in green circles ("differentiators") show angular deviations from discriminant axis (width of wedge = SEM interval): up = aligned, down = anti-aligned, sideways = off-domain effect. Colors and numbers indicate administration routes and doses, asterisks denote significantly non-zero classifier scores. Bold fonts denote reference compounds.

DISCUSSION: The domain profiles represented activation patterns with **high biological plausibility**. Profiles A and B comprised key areas which are believed to be strongly involved in mediating antipsychotic or antidepressant effects, respectively (e.g., mPFC = medial prefrontal cortex, OFC = orbitofrontal cortex, VTA = ventral tegmental area, RN = raphe nuclei, Th = thalamus, and others).[3,4] Profile C comprised only few ROIs, maybe because of the high diversity of MoAs among the reference compounds with little shared networks, maybe also confounded by interference between the benzodiazepines and the isoflurane anesthesia, both of which act on the GABAergic system. Nevertheless, the gauges **plausibly positioned** the vast majority of the pharmacological and even non-pharmacological treatments, with the differentiators providing additional valuable information on the relative contribution of **off-domain effects**. It appears that domains A, B and C may be best named as "1st/2nd generation antipsychotic (dopaminergic)", "serotonergic" and "benzodiazepine-like", respectively.

CONCLUSION: Upon judicious selection of domains and careful calibration of the gauges, our approach represents a valuable analytical tool for **concise quantitative characterization** and **biological interpretation** of drug-induced brain activation patterns. While establishing the domain profiles does require a considerable database, the approach is experimentally reasonably efficient in its application phase. Together with its perfectly **translational nature**, these aspects render it attractive for decision making in neuroscience drug discovery.

REFERENCES: **[1]** Jenkins, B.G. (2012): Neuroimage 62, 1072-1085. **[2]** Detre, J.A., et al. (1992): Magnetic Resonance in Medicine 23, 37-45. **[3]** Remington, G., et al. (2011): Expert Review of Neurotherapeutics 11, 589-607. **[4]** Phillips, M.L., et al. (2003): Biol Psychiatry 54, 515-528.