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Correlative analysis of dopaminergic and serotonergic metabolism across the brain to study monoaminergic function and interaction

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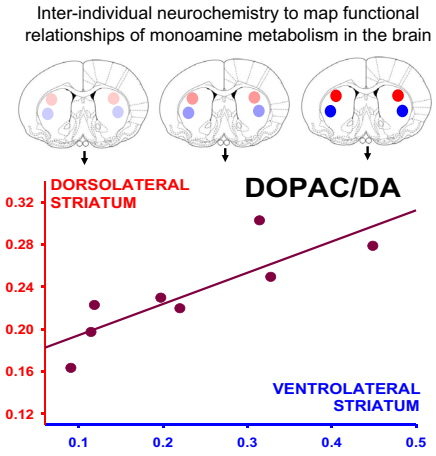
HIGHLIGHTS

- Monoamine and metabolite tissue contents often correlate within a brain region.
- Correlations between metabolites or turnovers are limited across brain regions.
- Dopamine turnovers correlate between striatal territories.
- Serotonin and dopamine turnovers in a same region positively correlate.
- A few relationships between dopamine and serotonin metabolites were found across brain regions.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: The widespread innervation of dopamine (DA) and serotonin (5-HT) systems in cortical and subcortical regions suggests that their biochemical interactions can occur in multiple regions directly or indirectly via neurobiological networks.
New method: The present study was aimed at validating a neurochemical approach of monoaminergic function based on inter-individual variability of monoamine tissue contents in various cortical and subcortical areas. We focused on monoamines metabolism and examined correlations within and between these monoaminergic systems in selected regions for the metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) and/or homovanillic acid (HVA) and 5-hydroxyindole acetic acid (5-HIAA) alone or with respect to the turnover indexes DOPAC/DA, DOPAC + HVA/DA and 5-HIAA/5-HT.
Results: The tissue content of metabolites and their parent drug correlated within a brain region. Conversely, a few specific relationships (10%) were observed for each turnover in paired brain regions and

Abbreviations: aCg, anterior cingulate cortex; aIns, anterior insular cortex; BLA, basolateral nucleus of the amygdala; CE, central nucleus of the amygdala; core, core of the nucleus accumbens; DA, dopamine; DLO, dorsolateral orbitofrontal cortex; DLS, dorsolateral striatum; DMS, dorsomedial striatum; DOPAC, 3,4-dihydroxyphenylacetic acid; DRN, dorsal raphe nucleus; HPC, hippocampus; HPLC, high pressure liquid chromatography; HVA, homovanillic acid; IL, infralimbic cortex; LO, lateral orbitofrontal cortex; M2, motor cortex M2; MO, medial orbitofrontal cortex; OFC, orbitofrontal cortices; pCg, posterior cingulate cortex; pIns, posterior insular cortex; PL, prelimbic cortex; 5-HIAA, 5-hydroxyindole-3-acetic acid; 5-HT, 5-hydroxytryptamine, serotonin; shell, shell of the nucleus accumbens; SNc, substantia nigra pars compacta; STN, subthalamic nucleus; VLS, ventrolateral striatum; VMS, ventromedial striatum; VTA, ventral tegmental area.

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Striatum
Linear regression
Correlation
Dopamine
Serotonin
HPLC
Electrochemical detection
Nucleus accumbens
Amygdala
Hippocampus
3,4-Dihydroxyphenylacetic acid
5-Hydroxyindole-3-acetic acid
Homovanillic acid
Rat

even less between the 5-HT and DA turnovers. The number of correlations was lower when looking at the metabolite tissue contents. In all cases, the 5-HT and DA turnover indexes or metabolites correlated positively within a brain region.
Comparison with existing method(s): These data validate the inter-individual analysis of monoamine tissue content by providing evidence that the metabolite correlates with the parent neurotransmitter in the same region. The pattern of correlations of metabolisms reported here differs from that of the parent neurotransmitters, notably regarding the relationships of DA turnovers between striatal territories.
Conclusion: The whole neurochemical approach is of interest for characterizing monoaminergic systems interaction in various genetic or pharmacological models of neuropsychiatric diseases.

1. Introduction

The serotonin (5-HT) and dopamine (DA) systems interact in the mammalian brain and regulate the activity of neurobiological networks (Dalley et al., 2011 De Deurwaerdere and Di Giovanni, 2016). Most forebrain structures receive terminals of 5-HT or DA neurons from midbrain raphe nuclei or from the ventral tegmental area and the substantia nigra, respectively (Björklund and Dunnett, 2007; Björklund and Lindvall, 1986; Hale and Lowry, 2011; Steinbusch, 1984). The pleiotropic action of these systems in the brain challenges the understanding of their biochemical interactions.

To address the widespread biochemical relationships of monoamines, we proposed an inter-individual approach in rats coupled to a correlative analysis of the tissue content of monoamines between twenty cerebral structures involved in cognition (Fitoussi et al., 2013). The tissue content for DA or 5-HT often correlated in the same regions and poorly at distal regions. Such an approach may inform about qualitative changes of monoamine tissue contents that could occur independently from quantitative changes as exemplified in the mussel *Perna Perna* (Klouche et al., 2015). However, the tissue content of the neurotransmitter has a limited functional value per se. It represents mostly the monoamine stored at high concentrations in different vesicular compartments which vary between regions (Commissiong, 1985; Eisenhofer et al., 2004; Glowinski, 1975; Green and Grahame-Smith, 1975). Due to the small size of the sampled structures, methodological caveats (heat, degradation, weight sample errors, sensitivity) might alter the quality of the analysis with almost no possibility of internal validation. Obtained values could be compared to those published in the literature but the later are often disparate and sometimes unknown for some brain regions.

The relationship between the content of the neurotransmitter and its metabolite should give a positive correlation of their tissue content within one region in the inter-individual analysis. This would be a good validation but the metabolism of monoamines is complex. It involves numerous enzymes including monoamine oxidases (MAO), aldehyde dehydrogenases and catechol-O-methyl transferases (COMT) specifically for the catecholamine dopamine. The DA metabolites 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 3-methoxytyramine and the 5-HT metabolite 5-hydroxyindole acetic acid (5-HIAA) are usually measured simultaneously with parent neurotransmitters and used because they are quite stable. None of these metabolites except 3-methoxytyramine, correspond to a direct compound with respect to the parent drug. Furthermore, the production of these metabolites obviously include other cells because COMT is not present in DA terminals (Schendzielorz et al., 2013) while MAO-A, which destroys 5-HT is more expressed outside 5-HT neurons (Youdim et al., 2006). The metabolism for a monoamine varies across brain regions as exemplified by the ratios DOPAC/DA [sometimes (HVA + DOPAC/DA)] and 5-HIAA/5-HT (Bannon et al., 1981; Commissiong, 1985; Ebinger et al., 1987; Fitoussi et al., 2013; Johnston and Moore, 1983; Shannon et al., 1986) whose functional value is more important than the tissue neurotransmitter itself (Eisenhofer et al., 2004). Altogether, the study of the metabolism would be a prerequisite to validate the inter-individual method. Beyond the validation of the whole study, the pattern of correlations of the metabolism could differ from that of the parent neurotransmitter previously reported (Fitoussi et al., 2013).

We took the previous neurochemical database obtained in various brain areas involved in cognition to extend the inter-individual approach of the 5-HT/DA interaction by including metabolite contents and indexes of turnover. In order to see the benefit of this complementary approach, we have specifically addressed (1) the relationship between the tissue content of metabolites with their neurotransmitter in a single brain region (2) the pattern of correlations of metabolites and turnovers within each neurotransmitter system between regions and (3) the pattern of correlations of metabolites and turnovers between the neurotransmitters systems in all sampled brain areas. This additional analysis on 5-HT/DA metabolism highlights the metabolic link in a single brain region between the neurotransmitter and its metabolite and underscores new patterns of correlations between regions and/or systems of neurotransmission that can be more pertinent than the sole measurement of tissue neurotransmitters.

2. Methods

The entire methodology has been published (Fitoussi et al., 2013) and it has been summarized and adapted herein.

2.1. Animals

Male Wistar rats (n = 35; Charles River, Lyon, France) weighing 400–600 g were kept at constant room temperature (21 ± 2 °C) and relative humidity (60%) with a 12-light/dark cycle (dark from 8 p.m.) and had free access to water and food. All animals use procedures conformed to European Economic Community (86-6091 EEC) and the French National Committee guidelines (*décret* 87/848, Ministère de l'Agriculture et de la Forêt) for the care and use of laboratory animals and were approved by the Ethical Committee of Centre National de la Recherche Scientifique, Région Aquitaine-Limousin. All animal experiments comply with the ARRIVE guidelines.

2.2. Tissue processing for histological verification and post-mortem analysis

Rats were kept in the vivarium for six weeks without any testing. They were gently taken outside the vivarium and immedi-