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Sociability trait and regional cerebral oxidative metabolism in rats: Predominantly nonlinear relations

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ABSTRACT

Deficits in social behaviour are common in psychopathological conditions e.g., depression, autism and schizophrenia. In rats, sociability, defined as the engagement of an animal in non-aggressive social contact with a conspecific in a neutral arena, is as a persistent trait. To elucidate the neuroanatomy of social behaviour in animal models, long term neuronal energy metabolism was studied in rats preselected for sociability levels. Rats were divided into groups with high, medium and low sociability levels (HS, MS and LS) according to the average score of three social interaction tests, and cerebral long-term energy metabolism was assayed with cytochrome oxidase histochemistry. In the dorsomedial caudate putamen oxidative metabolism was linearly dependent on sociability, with LS-rats having the highest levels. In median preoptic nucleus, posterior paraventricular thalamus and median raphe, nonlinear relations appeared, HS- and LS-rats having lower oxidative activity than MS-animals. In the supraoptic nucleus MS-rats displayed lower oxidative activity than HS- and LS-animals. Intra-individual variability in social interaction on different testing occasions correlated positively with oxidative metabolism in the prelimbic cortex, bed nucleus of stria terminalis and caudate putamen, and negatively in the nucleus accumbens core. Conclusively, rats with different sociability levels are distinguished by long-term energy metabolism in nuclei involved in motivational behaviour, fear and vigilance; the relationship between energy metabolism and sociability appears to be predominantly nonlinear – animals with high and low expression of sociability are similarly deviant from the average; and intra-individual variability in social interaction is related to brain areas controlling motivation, stress reactivity and anxiety.

1. Introduction

Disturbed social behaviour is symptomatic to a variety of psychopathological conditions, e.g., schizophrenia, autism spectrum disorders, depression and social anxiety disorder [1–6], thus social behaviour can serve as the core aspect or the readout in the corresponding animal models [7–12]. Inter-individual differences in rodent behaviour can reveal endophenotypes for the preclinical modelling of human disorders [13–15]. Rats differ in the levels of spontaneous social behaviour, and this can be quantified as time an animal spends in active social contact with a strange congener in social interaction. Repeated measurement of social behaviour reveals persistence of the animal in social situations, a stable sociability trait [16]. Sociability is expressed behaviourally as proneness to initiate or actively accept social contact with a conspecific on neutral territory. To assess the sociability trait, the social interaction (SI) test of anxiety can be repeatedly used [17,18], measuring social contact of a rat with different partners on 3–4 occasions. While there is significant intra-individual variability that probably reflects anxiety, and the behaviour is moderately correlated with the social activity of the partner, the average social activity over repeated testing is stable in time [16]. Response to chronic stress is dependent on sociability levels: Animals with high sociability levels developed anhedonic behaviour more readily after chronic stress, whereas low sociability rats showed increased forced swimming activity [19]. Thus sociability can be regarded as a stable phenotype involved in mediating stress-vulnerability/resilience.

According to our previous experiments [16,19], sociability is normally distributed in rats. In humans, both diminished sociability (e.g., in autism, depression, schizophrenia) and inappropriately high levels of sociability (e.g., Williams syndrome, manic episode in bipolar disorder) can be indicative of psychopathology [2,20–22]. Hence, for the study of the relationship between sociability and regional oxidative metabolism,

Abbreviations: BNST, bed nucleus of stria terminalis; COX, cytochrome oxidase; CP, caudate putamen; CPDM, dorsomedial caudate putamen; HS, MS and LS, high, medium and low sociability; SI test, social interaction test; MPN, median preoptic nucleus; MR, median raphe; NAcc, nucleus accumbens; PLC, prelimbic cortex; PVP, posterior paraventricular thalamic nucleus; SON, supraoptic hypothalamic nucleus

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we divided rats into high, medium and low sociability groups.

To broaden the scope of neuroanatomical networks studied in respect with social behaviour, metabolic mapping of energy expenditure can be applied [23]. Determining the long term neural activity of brain regions depending on the animals' sociability levels elucidates the neuroanatomical substrate of this trait. Neuronal function is almost entirely dependent on oxidative metabolism thus assessment of oxidative phosphorylation via the tissue levels of cytochrome c oxidase, the terminal electron transport complex with a key contribution to the chemo-osmotic gradient and ATP synthesis, reflects long-term neural activity [24–26].

The aim of this study was to identify brain regions with persistently different oxidative energy metabolism levels, as measured by cytochrome oxidase (COX) histochemistry, in rats with high, medium and low sociability, and to identify brain areas where oxidative metabolism is associated with intra-individual variability in social interaction levels.

2. Experimental procedures

2.1. Animals

Male Wistar rats (Scanbur BK AB, Sweden) were housed in standard cages in a light controlled room (12-h light/dark cycle; lights on at 8:30 a.m.) maintained at 22 °C with food and water available ad libitum. Animals were single-housed ten days before behavioural testing at the age of two months and sacrificed at three month of age. All experiments were in accordance with the EU Directive 2010/63/EU for animal experiments and approved by the Animal Experimentation Committee at the Estonian Ministry of Rural Affairs.

2.2. Social interaction test

Behaviour of rats (n = 28, weighing 370–435 g at the first behavioural test at 2 months of age) was measured in the social interaction (SI) test on three occasions separated by ten days. Sociability levels were based on the mean time in three SI tests spent in non-aggressive active social contact (allogrooming, sniffing the partner, crawling under and over, following) during a 10-minute session with a weight-matched strange rat on a 30 × 30 cm well-lit arena [16,19]. Based on the mean SI score animals with the highest (HS, n = 7), medium (MS, n = 8) and the lowest (LS, n = 8) sociability levels were chosen for histochemical analysis. Assessment of intra-individual variability of social interaction was based on the relative standard deviation of three SI tests (standard deviation/mean of tests*100).

2.3. COX histochemistry and image analysis

Sample handling, histochemical procedures and image analysis was carried out as previously reported [27,28], based on a modified protocol by Gonzalez-Lima and Cada [29]. Forty μm coronal sections were pre-incubated for 10 min with 0.0275% cobalt chloride (w/v) and 0.5% dimethyl sulphoxide (v/v) in 0.05 M Tris buffer with 10% sucrose (w/v) adjusted to pH 7.4 with 0.1% HCl (v/v). The sections were then incubated for one hour at room temperature in a solution consisting of 0.05% 3,3′-diaminobenzidine tetrahydrochloride (AppliChem), 0.0075% cytochrome c (Sigma), 5% sucrose, 0.002% catalase (Sigma) and 0.25% dimethyl sulphoxide (v/v) in sodium phosphate buffer (pH 7.4). Image analysis was conducted using the Image J 1.34 s freeware on the blue channel (resulting from a RGB split) of the background subtracted image. Eighty-seven brain regions were detected from the stained images with the help of rat brain atlas [30]. Optical density values were converted to enzyme activity levels by using external standardisation: Sections made of brain homogenate with spectrophotometrically measured enzyme activity were included in all incubation baths. In some cases the number of rats in a group for a given brain region varies because the CO activity was not measureable. This happened because of cutting-induced defects in brain slices or if the region was not obtainable due to the shift of the cut on the rostral-caudal axis.

2.4. Data analysis

All brain regions were analysed with ANOVA independently. Group-wise comparisons were carried out with Fisher LSD post hoc test. Normality of the distribution was assessed with the Shapiro-Wilk's test. Linear relationships between variables were assessed with Pearson's correlation test.

3. Results

The mean score of time spent in social interaction was normally distributed (W = 0.970, p = 0.6). The mean scores of SI tests were 46 ± 5.7, 87 ± 3.0 and 113 ± 5.3 (s, M ± SEM) for LS-, MS- and HS-animals respectively. The mean score of LS-rats differed significantly from MS-animals and the mean score of MS-rats differed significantly from HS-animals ($F_{1,28}$ = 50.1, p < 0.001, p < 0.001 and p < 0.001 respectively; LSD post hoc test).

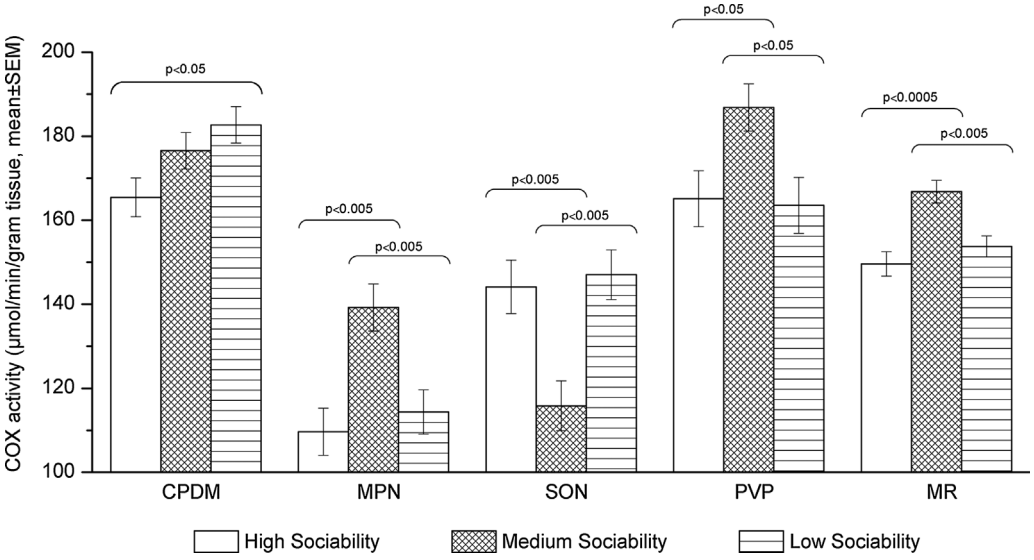

Fig. 1. Cytochrome oxidase activity (μmol/min/gram tissue, mean ± SEM) in animals with high, low and medium sociability levels. CPDM – dorsomedial caudate putamen, MPN – median preoptic nucleus, MR – median raphe PVP – posterior paraventricular thalamus, SON – supraoptic nucleus.