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Investigating the long-term effect of subchronic phencyclidine-treatment on novel object recognition and the association between the gut microbiota and behavior in the animal model of schizophrenia

B. Pyndt Jørgensen [a,*], L. Krych [b], T.B. Pedersen [d], N. Plath [c], J.P. Redrobe [c], A.K. Hansen [a], D.S. Nielsen [b], C.S. Pedersen [c], C. Larsen [a], D.B. Sørensen [a]

[a] Section of Experimental Animal Models, Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Thorvaldsensvej 57, 1871 Frederiksberg C., Denmark
[b] Department of Food Science, Faculty of Science, University of Copenhagen, Denmark
[c] Synaptic Transmission, H. Lundbeck A/S, Valby, Denmark
[d] Nonclinical Safety Research, H. Lundbeck A/S, Valby, Denmark

HIGHLIGHTS

- SubPCP impaired novel object recognition up to three weeks after washout.
- Alterations in the core microbiome suggested subPCP to affect the GM.
- GM profiles correlated to memory performance.
- Oral ampicillin treatment abolished the subPCP-induced cognitive deficit.
- The GM is suggested to impact cognition, contributing to variation within the model

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ABSTRACT

Subchronic phencyclidine (subPCP) treatment induces schizophrenic-like behavior in rodents, including cognitive deficits and increased locomotor sensitivity towards acute administration of PCP. Evidence is accumulating that the gut microbiota (GM) influences behavior through modulation of the microbiota–gut–brain axis, and hence, part of the variation within this animal model may derive from variation in the GM. The aims of this study was to investigate first, the duration of subPCP-induced cognitive impairment in the novel object recognition test, and second, the possible effect of subchronic PCP-treatment on the GM, and the association between the GM and the behavioral parameters. The association was further investigated by antibiotic reduction of the GM. Male Lister Hooded rats were dosed twice daily i.p. with either 5 mg/kg PCP or sterile isotonic saline for seven days followed by a seven-day washout period. Rats were tested in the novel object recognition and the locomotor activity assays immediately after, three weeks after, or six weeks after washout, and the fecal GM was analyzed by high throughput sequencing. Antibiotic- and control-treated rats were tested in the same manner following washout. In conclusion, subPCP-treatment impaired novel object recognition up to three weeks after washout, whereas locomotor sensitivity was increased for at least six weeks after washout. Differences in the core gut microbiome immediately after washout suggested subPCP treatment to alter the GM. GM profiles correlated to memory performance. Administration of ampicillin abolished the subPCP-induced memory deficit. It thus seems reasonable to speculate that the GM influences memory performance, contributing to variation within the model.

1. Introduction

The subchronic phencyclidine-induced animal model of schizophrenia mimics aspects of the NMDA receptor hypofunction hypothesis of schizophrenia, as reviewed by Javitt & Zukin and Jentsch & Roth [1,2], through administration of the non-competitive N-methyl-D-aspartase (NMDA) receptor antagonist phencyclidine (PCP). The model has the ability to induce schizophrenic-like behavior, modeling positive, negative and cognitive symptoms of the human [1–3], and it is therefore a frequently applied tool when testing new pharmacological candidates against the disease. To generate the model, rats are dosed with PCP twice a day for seven days, followed by a washout period of seven days, after which they exhibit schizophrenic-like behavior, such as

* Corresponding author.
E-mail address: bmp@sund.ku.dk (B. Pyndt Jørgensen).

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cognitive deficits [4,5] and increased locomotor sensitivity towards acute administration of PCP (aPCP) [6]. The increased locomotor activity in response to aPCP has previously been shown to be significantly increased up to 89 days after subchronic PCP-treatment [6], but the duration of subchronic PCP-induced cognitive impairment in the novel object recognition test (NOR) has never been investigated.

Moreover, variation exists within the model. As reviewed by Collins et al. [35], evidence is accumulating that the gut microbiota (GM) influences behavior through modulation of the microbiota–gut–brain axis (mGBA) [7–10]. Hence, part of the variation within the animal model may derive from variation in the GM [11]. The GM has previously been associated with memory performance [12–14] and locomotor activity [15], thus, the model-induced deficits within these behavioral parameters may possibly be affected by the GM, contributing to the variation within the model. Furthermore, subchronic PCP administration has been demonstrated to increase the stress response to a saline injection and a novel environment [16,17]. Stress affects gastrointestinal epithelial function, mucus secretion and motility [18], and hence, enhanced PCP-induced stress susceptibility may affect the gastrointestinal tract, secondly altering the GM composition. A previous study by Bendtsen et al. showed a stress-induced change in the GM of BALB/c mice, which was associated with an increase in anxiety [19], and a study by O'Mahony equally reported early life stress to alter the GM and behavior significantly [20]. A possible PCP-induced stress alteration in the GM may therefore affect behavior in the model.

The aims of this study were to investigate, first, the duration of subchronic PCP-induced cognitive impairment in the NOR test, second, the effect of subchronic PCP-treatment on the GM, and the association between the GM and the behavioral parameters measured. To further evaluate the influence of the GM on behavior, we repeated the study, including a vehicle- and a subPCP group in which the GM was substantially reduced by oral treatment with ampicillin through the entire study period. Behavior was evaluated in the NOR and the locomotor activity assay (MOTR).

2. Materials and methods

All studies were conducted in accordance with the EU directive 2010/63/EU, and the protocols were approved by the Danish Animal Experimentation Committee as required by the Danish Animal Experimentation Act (LBK 1306 from 23/11/2007 with 2011 amendments). Efforts were made to improve animal welfare and minimize stress of the rats, which were checked on a daily basis.

2.1. Long-term effect of PCP on memory and associations between gut microbiota and behavior

Three batches of 24 male Lister-Hooded rats (crl:LH, Charles River, Germany) with a weight of 100–130 g at arrival were housed in groups of four in Macrolon (type IV, Tecniplast, Scanbur A/S Denmark) cages equipped with sawdust bedding (aspen bedding, Tapvei, Brogaarden, Denmark), a plastic house (Rat Retreats™, Bio-Serv, USA), a wooden chew block (aspen bricks, Tapvei, Brogaarden, Denmark) and nesting material (Paper Wool, Scanbur A/S Denmark), and ad libitum access to water and standard rat chow (Altromin 1324, Brogaarden, Denmark). Lights were on from 6:00 a.m. in a 12-hour light/dark cycle, and humidity and temperature were 30%–70% and 20 ± 2 °C, respectively. Batches were acclimatized for 12 days before the cages were randomly assigned to either PCP or vehicle treatment (3 cages in each group), and dosed IP with either 5 mg/kg free base PCP (1 ml/kg, synthesized in-house) or sterile isotonic saline water (1 ml/kg) at 7.00 a.m. and 7.00 p.m. for 7 days, followed by a washout period of 7 days, as previously described [5]. Rats were then tested in the NOR, left alone for one day and then in the MOTR with a timespan after the washout period of 0 (T0), 3 (T3) and 6 (T6) weeks. At euthanasia rats were anesthetized by the use of Avertin (1 ml/100 g) (Tribromoethanol, synthesized in-house) before the rats were euthanized by total bleeding. Fecal samples were obtained after completion of the NOR to avoid bias by an acute stress effect on the GM in the MOTR. Fig. 1 illustrates the timeline of the study.

2.1.1. Novel object recognition test

The test was performed as previously described [5]. Briefly, rats were habituated to the test room for 24 h before testing. The test was performed in a dimly lit box of the size 40 × 60 cm. At day one, the rats were habituated to the box together with their cage mates twice for 10 min with a four-hour interval. At the second day, acquisition and test trials were performed. Rats were placed in the box alone for acquisition trial and allowed to investigate two similar objects for 3 min before being returned to their home cage. After a one-hour, inter-trial interval the rats were placed in the box again for the test trial, and allowed to investigate the familiar object and a novel object for another 3 min. The objects were a globe-formed, clear glass, paper dome with a blue color in the center (diameter of 8 cm) and an opaque Perspex pyramid (10 × 10 × 6 cm, made in-house) placed in the center of the two halves of the box. The objects were cleaned with 70% ethanol between each trial. The box was wiped with a dry paper towel between each trial, and feces and urine were removed with a wet paper towel. Animals were at cage level randomized to the different novel objects and their left/right position. The acquisition and the test trials were video recorded and scored blinded manually afterwards. Exploration was defined as sniffing, licking and touching the object while facing it. Acquisition requirements were 20 s spent investigating the two similar objects, with a minimum of 2 s spent on a single object. Test requirements were a minimum of 15 s exploring the objects, with a minimum of 2 s spent on a single object. In the test trial the difference between time spent investigating the novel and the familiar object divided by the total time spent investigating the objects was calculated as a measure of memory performance (Discrimination index, DI). A high score

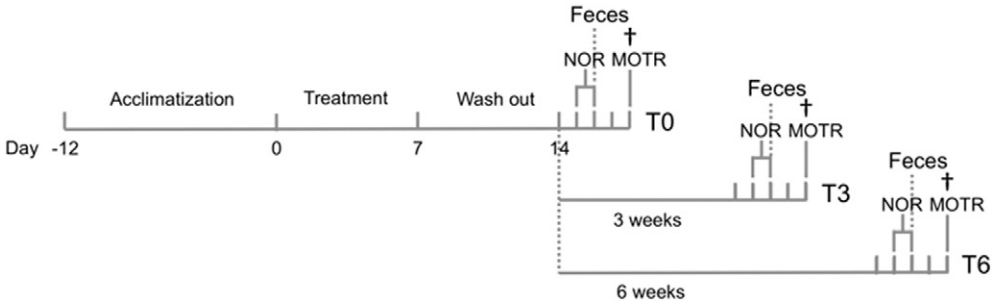

Fig. 1. Timeline of the study. Three batches were acclimatized, subjected to the subchronic PCP-dosing regimen, receiving either PCP or saline (vehicle) twice a day for seven days, followed by a washout period of seven days. Batches were then tested in the novel object recognition (NOR) and the locomotor activity assay (MOTR) at 0 (T0), 3 (T3) and 6 (T6) weeks after washout and euthanized immediately after the last test. †: Euthanasia.