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# Serotonin depletion induces pessimistic-like behavior in a cognitive bias paradigm in pigs

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## HIGHLIGHTS

- *para*-Chlorophenylalanine depletes the brain of serotonin for at least 13 days.
- The effects of serotonin depletion were observed in behavioral tests.
- Serotonin depletion induced pessimistic-like behavior indicating negative affect.
- Behavior in an open field/novel object test showed no consistent treatment effects.

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## ABSTRACT

Cognitive and affective processes are highly interrelated. This has implications for neuropsychiatric disorders such as major depressive disorder in humans but also for the welfare of non-human animals. The brain serotonergic system might play a key role in mediating the relationship between cognitive functions and affective regulation. The aim of our study was to examine the influence of serotonin depletion on the affective state and cognitive processing in pigs, an important farm animal species but also a potential model species for biomedical research in humans. For this purpose, we modified a serotonin depletion model using *para*-chlorophenylalanine (pCPA) to decrease serotonin levels in brain areas involved in cognitive and affective processing (part 1). The consequences of serotonin depletion were then measured in two behavioral tests (part 2): the spatial judgement task (SJT), providing information about the effects of the affective state on cognitive processing, and the open field/novel object (OFNO) test, which measures behavioral reactions to novelty that are assumed to reflect affective state. In part 1, 40 pigs were treated with either pCPA or saline for six consecutive days. Serotonin levels were assessed in seven different brain regions 4, 5, 6, 11 and 13 days after the first injection. Serotonin was significantly depleted in all analyzed brain regions up to 13 days after the first application. In part 2, the pCPA model was applied to 48 animals in behavioral testing. Behavioral tests, the OFNO test and the SJT, were conducted both before and after pCPA/saline injections. While results from the OFNO tests were inconclusive, an effect of treatment as well as an effect of the phase (before and after treatment) was observed in the SJT. Animals treated with pCPA showed more pessimistic-like behavior, suggesting a more negative affective state due to serotonin depletion. Thus, our results confirm that the serotonergic system is a key player in cognitive-emotional processing. Hence, the serotonin depletion model and the spatial judgement task can increase our understanding of the basic mechanisms underlying both human neuropsychiatric disorders and animal welfare.



## 1. Introduction

Modern theories describe the brain as a complex network with a high connectivity between different brain regions jointly regulating affective or cognitive functions [1]. Affective states, i.e. short-term emotions and long-term moods, therefore, can modulate cognitive processes and vice versa. A variety of neuropsychiatric disorders are reflected by changes in both affect and cognition. Patients suffering

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from major depressive disorder show a so-called 'negative or pessimistic cognitive bias', meaning for example a tendency to interpret ambiguous stimuli as more pessimistic than healthy individuals [2]. However, the physiological mechanisms underlying such interactions between affect and cognition are largely unknown [3]. One prominent transmitter system of interest in affective and cognitive research is the serotonergic system. Serotonin (5-HT) plays a crucial role in cognitive functions, for example in memory consolidation [4] and learning [5]. On the other hand, 5-HT was found to regulate affective states [6], such as anxiety [7] and aggression [8], and is known for its relevance in major depressive disorder [9]. Serotonergic neurons originate from the raphe nuclei of the brainstem, and its projections innervate multiple brain structures related to mood disorders and processes of attention towards stimuli, stimulus evaluation and (selection of) behavioral responses to these stimuli, such as the amygdala [10], anterior cingulate cortex [11], striatum [12], hippocampus [13], hypothalamus [14], and prefrontal cortex [15]. Therefore, a pharmacological 5-HT depletion model using *para*-chlorophenylalanine (pCPA) has been developed to study the biobehavioral effects of serotonin ([16]; for review see [17]). PCPA is an inhibitor of tryptophan hydroxylase, the rate-limiting enzyme in 5-HT biosynthesis. It was successfully used for selective 5-HT depletion in different species such as rats [18], sheep [19] and, recently, in pigs [20].

A number of behavioral tests have been applied to evaluate the affective state in non-human animals (for review see [21]). Here, the focus lies on the implications for animal welfare. This calls for experimental designs to specifically test the valence dimension of affective states (e.g., [22]). The most promising approach is the cognitive bias test, which is well established in human psychology (see above) and measures affective valence indirectly by observing its effect on cognitive processing. Harding et al. [23] first established a nonverbal paradigm for testing cognitive judgement bias for use in animal experiments. Their approach is based on a discrimination task, where animals learn to distinguish two stimuli with outcomes of different valence. The information on the stimulus-response-outcome contiguity then has to be transferred to judge the most likely outcome of novel, ambiguous stimuli. Current research implies that affective states influence this judgement [24]. The approach has been used in a variety of non-human animals, for example in rats [25], monkeys [26] and, recently, in pigs [27–30]. Recently, pharmacological manipulation of the 5-HT system was shown to alter the behavioral response in the cognitive bias task in sheep [19] and rats [31]. McHugh et al. [32] showed that the serotonin transporter in mice, similar to in humans [33], modulates the behavioral response in cognitive bias tasks. As opposed to these approaches, the combined open field/novel object (OFNO) test, where animals are first confronted with a novel environment and then with an unfamiliar object, involves less (or less complex) cognitive processing. The OFNO test rather measures behavioral reactivity (e.g., [34–36]), i.e. it scores affective behavior patterns in response to an emotionally challenging situation. Thus, it has been suggested that this kind of emotional reactivity primarily relates to the arousal dimension [37]. Alterations in 5-HT availability in the brain have been shown to change behavior in the OFNO test in different species. In rats, a knockout of the 5-HT2 receptor resulted in less anxiety-like behavior [38]. Moreover, 5-HT depletion decreased the vocalization rate in sheep, supporting the suggestion of diminished emotional reactivity of the animals [19].

The aim of the present study was to investigate the effects of central 5-HT depletion on affective states and cognitive processing in pigs. Thus, we established and applied a 5-HT depletion model using pCPA to decrease 5-HT levels in various brain regions involved in processes of attention towards stimuli, stimulus evaluation and (selection of) behavioral responses to these stimuli as well as affective processing. Using this model, we then investigated the impact of 5-HT depletion on two behavioral tasks, one of them providing information about behavioral reactivity (OFNO) and one measuring the interaction of affective and cognitive processing (cognitive bias). For the latter, we have previously developed a reliable design for eliciting stable behavioral responses of pigs in a repeated spatial judgement task (SJT; [29]). We hypothesized that 5-HT depletion would result in a behavioral alteration in both tests, indicating a negative affective state. In the OFNO test, we predicted more depressive-like behavior in the pCPA group (combination of decreased emotional reactivity/low arousal level and neophobia), which would be indicated by an increased latency in approaching the novel object [37] as well as a decreased activity [36]. In the SJT, we hypothesized a more pessimistic-like interpretation of ambiguous stimuli due to 5-HT depletion. We suggest that our approach will provide valuable insights into neurobehavioral processes underlying animal welfare in an important farm animal species. These results obtained with the pig, a model animal species for humans (e.g., [39, 40]), may also have implications for biomedical research.

## 2. Materials and methods

### 2.1. Subjects and housing

The study was conducted in two parts: first, the verification of the 5-HT depletion model ($n = 40$) and second, the application of the model in two behavioral tests, the spatial judgement task and the open field/novel object test ($n = 48$). Both parts were conducted in the experimental pig unit of the Leibniz Institute for Farm Animal Biology with pre-pubertal, female German Landrace piglets (36 days of age at the start of the experiments, 71 days at the end) bred there for experimental purposes. The studies were conducted in several replicates (two for part one, six for part two). In order to standardize group compositions within replicates, we selected 20 animals per replicate from a larger pool of animals. These originated from 5 different litters, and four animals were taken from each of these five litters. These were then pseudo-randomly assigned to two groups, splitting the four full-siblings of a litter into two pairs. Groups were established after weaning (28 days of age) and remained stable throughout experiments.

Pens measured 1.7 × 2.5 m. Commercial piglet food (Trede und von Pein, Itzehoe, Germany) and water were provided *ad libitum*. Room temperature was automatically controlled, starting at 28 °C and decreasing stepwise to 19 °C on the final experimental day to meet the changing requirements of the animals with age (Euromatic temperature curve). Pens were enriched with commercial and custom-made pig toys, and the animals received a mix of chopped straw, hemp pellets and sawdust (approx. 50 g) twice a day.

### 2.2. Part 1: verification of the serotonin depletion model

#### 2.2.1. Experimental design

The study was conducted in two replicates between November 2013 and January 2014 with 20 animals per replicate ($n_{total} = 40$). Starting at 56 days of age, pigs were injected i.p. with either 50 mg/kg pCPA (4-chloro-DL-phenylalanine, Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) dissolved in approx. 10 ml saline (pCPA group) or 10 ml saline (control group) at 14:00 h for up to six consecutive days (Fig. 1). Concentrations of 5-HT were analyzed in seven brain areas (see Section 2.2.2) at five time points corresponding to testing days in part two of this study (days 4, 5, 6, 11 and 13 after the first injection). Per time point, eight animals (four per replicate) were sampled. Subjects were assigned to treatment groups and time points of brain sampling pseudo-randomly, balanced for housing group and kinship.

#### 2.2.2. Brain tissue collection

Animals were narcotized with Ursotamin (ketamine, 10 mg/ml; Serumwerk Bernburg AG, Bernburg, Germany) and Stresnil (azaperone, 40 mg/ml; Janssen-Cilag GmbH, Neuss, Germany) and afterwards, were sacrificed with i.v. injections of T61 (embutramide, 200 mg/ml; mebezonium iodide, 50 mg/ml; tetracaine hydrochloride, 5 mg/ml; Intervet Deutschland GmbH, Unterschleißheim, Germany). Doses were adjusted to body weight as instructed by the manufacturers.