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Prenatal exposure to methadone or buprenorphine impairs cognitive performance in young adult rats

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ABSTRACT

Background: Concerns have been raised about the use of opioid maintenance treatment (OMT) during pregnancy and negative effects for the offspring. While neonatal outcomes and short-term effects are relatively well described, studies examining long-term effects in adolescents and adults are absent. The aim of the present study was to examine effects on learning and memory in young adult rats prenatally exposed to methadone or buprenorphine.

Methods: Female rats were implanted with a 28-day osmotic minipump delivering methadone (10 mg/kg/day), buprenorphine (1 mg/kg/day) or vehicle 5 days prior to mating. To examine possible effects on cognitive functioning, young adult offspring were included in three different behavioral tests that examine recognition memory, nonspatial, and spatial learning and memory. In addition, offspring growth and maternal behavior after birh were investigated.

Results: Prenatal exposure to methadone or buprenorphine caused impaired recognition memory and nonspatial reference learning and memory in young adult rats compared with the vehicle-treated group. Methadone-exposed offspring, but not the buprenorphine-exposed, also showed reduced long-term spatial memory. We did not observe any changes in maternal behavior or offspring growth after prenatal exposure to methadone or buprenorphine, suggesting that the impaired cognitive functioning is due to the opioid exposure rather than reduced maternal caregiving.

Conclusion: The present findings of long-term cognitive impairments in methadone- and buprenorphine-exposed offspring points to a negative impact of OMT on neurobiological development.

1. Introduction

Opioid maintenance treatment (OMT) is the recommended therapy for opioid dependence, also during pregnancy (WHO, 2014). While substitution treatment with methadone or buprenorphine has considerable benefits for the mother (Minozzi et al., 2013), there is a growing concern that the brain development of the fetus may be negatively affected. A majority of the babies born to mothers in OMT show signs of the neonatal abstinence syndrome (Welle-Strand et al., 2013; Wurst et al., 2016), are born preterm (Cleary et al., 2012; Lemon et al., 2018) and small for gestational age (Cleary et al., 2012; Norgaard et al., 2015), and have reduced head circumference compared with nonexposed children (Bier et al., 2015; Mactier et al., 2014). Furthermore, an increasing number of studies have reported negative neurobiological effects in preschool children, including impaired cognitive functioning, psychomotor impairments and attention and behavioral problems (de Cubas and Field, 1993; Konijnenberg and Melinder, 2015a; Sundelin Wahlsten and Sarman, 2013).

To date, there is a lack of knowledge concerning OMT during pregnancy and how the children manage when they enter adolescence and adulthood. The only study that has investigated school-aged children and youths exposed to methadone during fetal life reported an array of deficits in cognitive, perceptual-motor and behavioral functioning; but, this study contained several methodological limitations that might have influenced the results as discussed by the authors (Davis and Templer, 1988). Based on the more favorable birth

Abbreviations: OMT, opioid maintenance treatment; PND, postnatal day; SBD, simultaneous brightness discrimination; MWM, Morris water maze; s.c., subcutaneous

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outcomes compared with methadone (Jones et al., 2010; Meyer et al., 2015; Welle-Strand et al., 2013; Zedler et al., 2016), buprenorphine is presently recommended as the first choice for OMT during pregnancy. However, long-term outcomes after prenatal buprenorphine exposure have hardly been investigated.

Possible causal relationships between prenatal methadone or buprenorphine exposure and long-term effects are difficult to reveal in human observational studies, as OMT is accompanied by multiple risk factors that might affect the child, including maternal polysubstance use (Dorrie et al., 2014; England et al., 2017), impaired nutritional status (Marques et al., 2013; Tous et al., 2019), increased stress (Monk et al., 2012), and detrimental postnatal care environment (Fisher, 2015; Ornoy et al., 2010). Since experimental studies with methadone or buprenorphine cannot be performed in pregnant women due to obvious ethical considerations, controlled animal studies are valuable as they provide data not influenced by numerous confounders. Previous animal studies on neonatal outcomes following prenatal methadone or buprenorphine exposure have shown effects that closely resembled those reported in children born to mothers in OMT (Barr et al., 1998; Hutchings et al., 1979; Kongstorp et al., 2019; Kunko et al., 1996; Robinson et al., 1991; Robinson and Wallace, 2001).

In the present study, we examined the long-term effects of prenatal methadone or buprenorphine exposure on cognitive functioning in rats. To address this, we used an exposure regimen that provided blood concentrations of methadone or buprenorphine in pregnant rats comparable to those reported in pregnant women in OMT (Bartu et al., 2012; Concheiro et al., 2010; Gordon et al., 2010; Kongstorp et al., 2019). When the offspring reached young adulthood, their cognitive performance was tested using three different behavioral tests specific for recognition memory, nonspatial, and spatial learning and memory.

2. Materials and methods

2.1. Animals

The studies were approved by the Norwegian Animal Research Authority (Project ID: 9464; Norwegian Food Safety Authority, Oslo, Norway) and performed in accordance with the ARRIVE (Animals in Research: Reporting in Vivo Experiments) (Kilkenny et al., 2010) and the laws and regulations controlling experiments and procedures on research animals in Norway. Female (346.9 ± 8.1 g, n = 55) and male (12 weeks, n = 28) Sprague-Dawley rats were delivered by Taconic (Ejby, Denmark). Upon arrival, the animals were housed 2–4 per cage with regulated temperature, humidity and light (21 ± 2 °C, 50 ± 10% humidity, light period 8 am–8 pm), and food and water available *ad libitum*. All animals were allowed at least one week to habituate before being included in the experiments. The maternal weight, neonatal outcomes (e.g. body weight and length) and withdrawal symptoms (ultrasonic vocalizations) from animals included in this study have been reported previously (Kongstorp et al., 2019).

2.2. Drugs

Methadone − HCl (MW 345.91; Sigma, Oslo, Norway) and buprenorphine − HCl (MW 504.11; Chiron AS, Norway) were dissolved in sterile water. Sterile water was used as vehicle due to the low solubility of buprenorphine in saline.

2.3. Maternal exposure and mating

The female rats were randomly assigned to one of three different treatment groups: vehicle (sterile water; n = 16), methadone (10 mg/kg/day; n = 16) or buprenorphine (1 mg/kg/day; n = 23). The drugs were administered via osmotic minipumps and the doses were based on previous studies (Chen et al., 2015; Hung et al., 2013; Hutchings et al., 1992; Robinson and Wallace, 2001). This exposure regimen has been shown to induce stable blood concentrations of 0.25 ± 0.02 μM methadone and 5.65 ± 0.16 nM buprenorphine in the pregnant rats, which are comparable to the concentrations reported in pregnant women in OMT (Bartu et al., 2012; Concheiro et al., 2010; Gordon et al., 2010; Kongstorp et al., 2019).

As described in Kongstorp et al. (2019), female rats were implanted with a 28-day osmotic minipump (2ML4; Alzet, Cupertino, CA). Under isoflurane anesthesia (Baxter, Deerfield, IL), a small lateral incision was made in the dorsal area behind the neck. The pump was inserted subcutaneously (s.c.) with the opening facing in the anterior direction, and the incision site was closed with nonabsorbable stitches. After surgery, the animals were allowed to recover under observation on heating pads before being placed in individual cages. Metacam (0.3 mg/kg; s.c.) was given as an analgesic during and 5 − 6 h after surgery.

Five days after pump implantation, the females were mated with drug-naïve males (one male to one female). To avoid the possible influence of maternal stress on subsequent offspring behavior, the dams were left undisturbed throughout gestation. By the time of delivery the initial doses of 10 mg/kg/day methadone and 1 mg/kg/day buprenorphine were reduced to approximately 8 and 0.8 mg/kg/day, respectively, due to the maternal weight gain (Kongstorp et al., 2019).

2.4. Offspring growth

A total of 275 offspring (males and females) were included in the present study. The day of birth was noted as postnatal day (PND) 0. On PND 3, two randomly chosen offspring from each litter were weighed. All pups were examined for body weight and body length on PND 7, 14 and 21. Offspring were weaned and housed 2–3 of the same sex per cage on PND 21.

2.5. Maternal behavior

On PND 5, maternal behavior was examined. The nesting material was removed, and the home cage was placed under dimmed lighting. The behavior was recorded for 60 min by an overhead video camera (Noldus Information Technology, Wageningen, The Netherlands) to measure nursing (attendance with the pups), nesting, exploration, self-grooming and resting. The recordings were scored by a researcher blinded to the treatment of the dam.

2.6. Behavioral tests

The behavioral testing was conducted in 7- to 10-week-old offspring during the light period of the day under dimmed lighting. The rats were randomly assigned to the three different behavioral tests: (1) the novel object recognition test, (2) the simultaneous brightness discrimination (SBD) test, and (3) the Morris water maze (MWM) test. A maximum of 4 offspring (1 − 2 males and 1 − 2 females) from the same litter were included in each of the behavioral tests, and each animal was counted as n = 1. The majority of the animals (n = 89) were included in one behavioral test, while some animals (n = 29) were included in both the novel object recognition test and the MWM test. For two weeks before the behavioral testing, all offspring were handled for at least two min daily, which included individual exploration of a table top. All behavioral tests were performed by a researcher blinded to the treatment of the animals.

2.6.1. Novel object recognition test

The offspring were assessed in the novel object recognition test at 8 weeks of age to investigate recognition memory. The test was performed as previously described (Andersen et al., 2011). The apparatus consisted of a Plexiglas cage (56 × 34 × 20 cm) where the floor was marked with 18 equal squares (9 × 11 cm). The day before testing, each animal was allowed to explore the empty test cage for 15 min.

The test consisted of two phases. In phase 1, three identical

aluminum cubes (5 × 5 × 5 cm) were placed at fixed positions evenly distributed in the cage, serving as neutral objects. Each rat was allowed 5 min to explore the test cage and the neutral cubes. The animal was then placed back in its home cage for 10 min. The test apparatus was cleaned, and the center neutral object was replaced with a smaller cube (4.5 × 4.5 × 4.5 cm) with two uneven sides, which served as the novel object. In phase 2, the rat was allowed 5 min to explore the cage with the novel and the two neutral objects.

During each phase, the following behaviors were recorded: object exploration (seconds exploring each object), total exploration, total locomotion (number of squares crossed) and number of rearings. The exploration of an object was noted when the animal's snout was directed towards the object at a distance of 1 − 2 cm or less. Novelty preference was scored as the difference in time exploring the novel object versus the mean time exploring the neutral objects during phase 2. The apparatus was cleaned between each animal to avoid olfactory cues.

2.6.2. Simultaneous brightness discrimination (SBD) test

The SBD test was performed in 7-week-old offspring to examine nonspatial reference learning and memory. The test was performed as previously described (Myhrer and Naevdal, 1989). The apparatus consisted of a Plexiglas cage (56 × 34 × 20 cm) divided into two equal compartments (start and goal compartment) by a Plexiglas wall with an opening (10 × 10 cm) in the middle. The day before testing, each animal was allowed to explore the empty test cage for 15 min. During testing, three interchangeable aluminum cylinders (3 × 7 cm, black or light gray) with a round well (diameter: 2 cm) on the top were located in fixed positions in the goal compartment and served as discriminators. Two cylinders were designated as negative (same color), and one cylinder was designated as positive (different color). The well of the positive cylinder was filled with water. The positive cylinder was black for 50% of the animals and light gray for 50% of the animals. The position of the positive cylinder (right, middle or left) was changed between each trial according to a prearranged randomized order. The animals were deprived of water for 23 h prior to testing.

The test was divided into two days of acquisition (days 1 and 2) and a retention test (day 15). On the first day of acquisition (day 1), the rat was given 10 trials to learn to discriminate between the cylinders. When the rat encountered the positive cylinder, it was allowed a few laps of water as a reward and then placed in its home cage for 20 s before the next trial. On the second day of acquisition (day 2), the animal had to perform 5 correct trials in succession to achieve the learning criterion. A maximum of 20 trials were set. The retention test was performed on day 15, using the same criterion as on day 2.

The number of trials and errors to reach criterion were recorded on each day. To drink or investigate whether the well of a cylinder contained water, the animal had to stand on its hind legs with at least one forepaw on the top of the cylinder. Error responses were scored when a negative cylinder was mounted and an empty well was found. The apparatus was cleaned between each animal to eliminate olfactory cues.

2.6.3. Morris water maze (MWM) test

At 10 weeks of age, the offspring were included in the MWM test to examine spatial learning and memory. The test was performed as previously described (Morris, 1981), with some modifications. The apparatus consisted of a black circular pool (diameter: 180 cm; Noldus Information Technology) filled with water (22 ± 2 °C). Four starting points around the circumference of the pool were designated as north (N), south (S), east (E), and west (W), dividing the pool into 4 equal quadrants (NE, NW, SE and SW). A platform of clear plastic was placed at a fixed position in the NE quadrant with the top 1 − 2 cm below the water surface, making it invisible to the swimming rat. Distal visual cues were placed on the surrounding walls. All trials were recorded by an overhead video camera (Noldus Information Technology). During the two days before testing started, each rat was allowed to swim in the pool without the platform for 60 s/day.

The MWM experiment consisted of an acquisition phase (day 1–4), retention tests (day 5 and 12) and a visual platform test (day 12). During acquisition, 4 daily trials (two in the morning (starting at 9 am) and two in the afternoon (starting at 1 pm)) were performed for 4 consecutive days. The rat was placed in the water facing the wall at one of the four starting positions and allowed to swim for 60 s or until the platform was found. If the platform was not found, the animal was gently guided to the platform. The animal had to remain on the platform for 15 s at the end of each trial. The starting position was different for each trial according to a prearranged randomized order. Escape latency (time to locate the hidden platform), travel distance, and swimming velocity were recorded.

During the retention tests, the platform was removed. The rat was placed in the pool facing the wall at the S or W position (in a counterbalanced order) and allowed to swim for 30 s. The time spent in the NE quadrant was recorded.

After retention testing on day 12, the visual platform test was performed. The platform was placed in the NE quadrant, with the top 2 cm above the water surface, making the platform visible. The animal was placed in the middle of the S and W starting position, facing the wall. The time used to escape to the visible platform was recorded.

2.7. Data and statistical analysis

All data are presented as the mean ± SEM and individual data points unless stated otherwise. The maternal behavior and the MWM test were analyzed using Ethovision XT 11.0 tracking software (Noldus Information Technology). For comparisons of the three treatment groups, two-way analysis of variance (ANOVA) was used. In Figs. 2–4, treatment and sex were set as factors. A p-value below 0.05 was considered statistically significant. When the ANOVA revealed a significant effect, the Dunnett's post hoc test was used for individual group comparisons. The statistical analyses were conducted in SPSS version 25 (SPSS, Chicago, IL).

3. Results

A total of 55 female rats were implanted with an osmotic minipump delivering methadone, buprenorphine or vehicle. Seven animals were euthanized during the experiment due to health problems or complications with the pump (e.g. opening of incision site or inflammation). Six vehicle-exposed, 2 methadone-exposed, and 10 buprenorphine-exposed females were not successfully mated. There were no significant differences in mating success, litter size, number of stillborn offspring or sex ratio, which have been reported previously (Kongstorp et al., 2019). Moreover, the number of offspring found deceased during the first week after birth did not differ between the three treatment groups (data not shown).

3.1. Maternal behavior and offspring growth

Maternal behavior, measured as time spent nursing, nesting, exploring, self-grooming and resting, was not affected by gestational exposure to methadone or buprenorphine when examined five days after birth (Fig. 1A). No significant differences were observed in the offspring body weight or body length during lactation neither when litters (Fig. 1B and C) nor individual pups (not shown) were compared.

3.2. Recognition memory

Prenatal exposure to methadone or buprenorphine significantly reduced the preference for novelty in young adult rats [$F_{(2,42)} = 9.20$, $p < 0.001$]. The novelty preference among the methadone- and buprenorphine-exposed animals was reduced by 73 ± 13 % and 84 ± 11 %, respectively, compared with the vehicle-exposed group

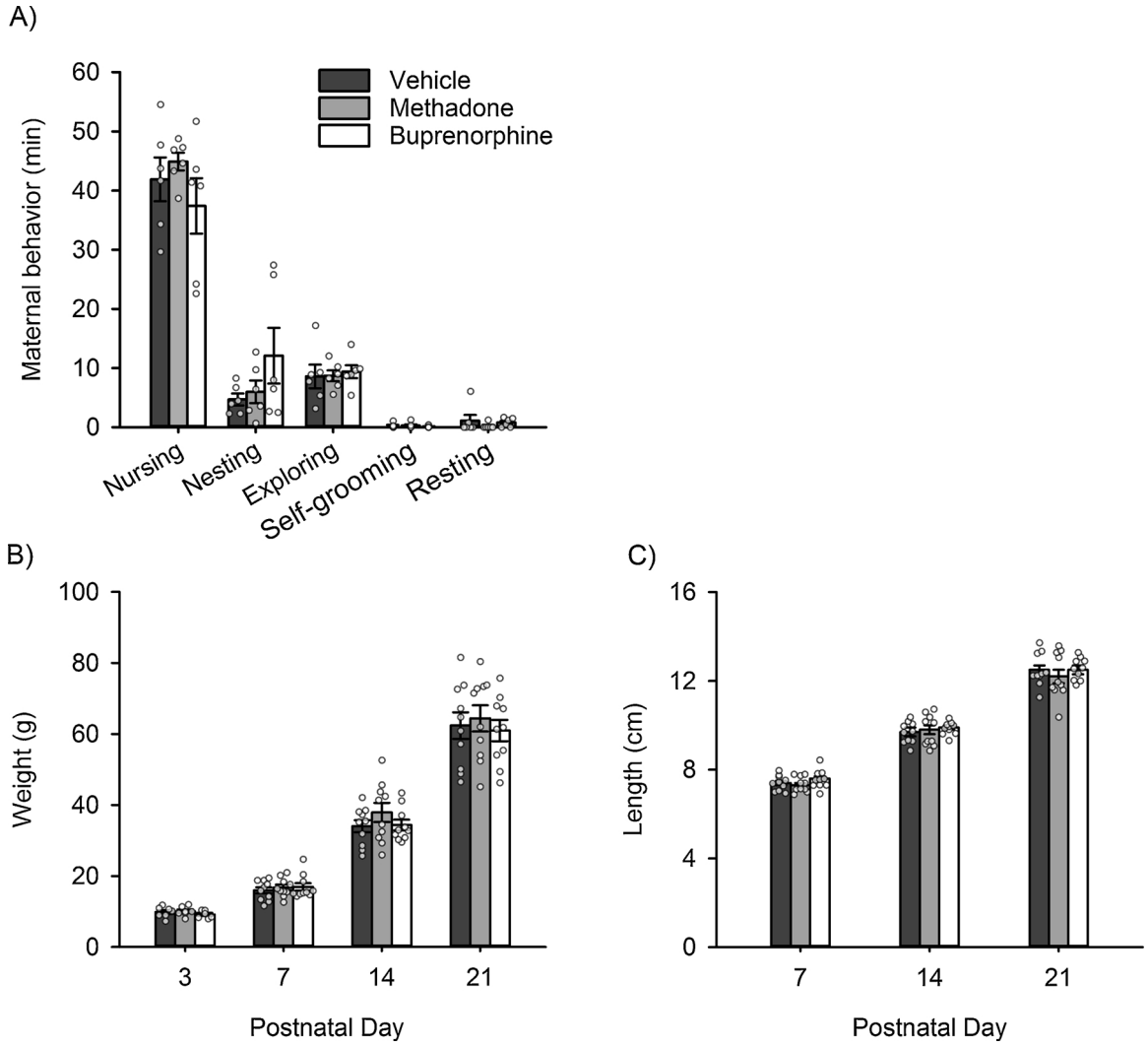

Fig. 1. Exposure to methadone (10 mg/kg/day) or buprenorphine (1 mg/kg/day) during gestation did not affect maternal behavior or offspring growth. (A) Maternal behavior measured for 60 min five days after birth. (B) Offspring body weight (g) and (C) body length (cm) measured on postnatal day 3, 7, 14 and 21. The results are shown as mean ± SEM and individual data points. Maternal behavior; n = 6 dams, offspring body weight and length; n = 6-10 litters. No significant differences were revealed, two-way ANOVA.

($p < 0.01$; Fig. 2A). The exploration of the neutral objects was similar for all the three groups in both phase 1 (data not shown) and phase 2 (Fig. 2A). However, the methadone- and buprenorphine-exposed animals tended to spend less time exploring the novel object compared to the vehicle-exposed animals [$F_{(2,42)} = 2.65$, $p = 0.08$], resulting in a significantly reduced novelty preference. No differences were observed in the total exploration time, the total locomotor activity, or the number of rearings (Fig. 2B–D). Statisical analyses (two-way ANOVA) did not reveal any significant differences between males and females, or any interaction between treatment and sex.

3.3. Nonspatial reference learning and memory

Prenatal exposure to buprenorphine resulted in impaired nonspatial reference learning in young adult rats [$F_{(2,36)} = 3.74$, $p = 0.03$]. While the vehicle-exposed offspring took 7.8 ± 0.6 trials to reach the criterion (5 correct trials in succession) on the second day of acquisition, the buprenorphine-exposed animals took 12.9 ± 1.6 trials ($p < 0.05$; Fig. 3A). The methadone-exposed offspring showed a tendency to use a higher number of trials (11.9 ± 1.3) on the second day of acquisition ($p = 0.06$; Fig. 3A). Prenatal opioid exposure also impaired nonspatial reference memory, examined in the retention test on day 15 [$F_{(2,36)} = 8.02$, $p = 0.001$]. The methadone- and buprenorphine-exposed rats took 12.5 ± 1.4 and 12.3 ± 1.5 trials, respectively, to reach the criterion, both of which were significantly higher than the 6.4 ± 0.8 trials taken by the vehicle-exposed animals ($p < 0.01$; Fig. 3A). The number of errors made during acquisition did not differ significantly between the three groups (Fig. 3B). During the retention testing on day 15, however, a significant effect of treatment was revealed for the number of errors made [$F_{(2,36)} = 2.40$, $p = 0.009$]. The methadone- and buprenorphine-exposed rats made 6.0 ± 1.4 and 5.5 ± 1.7 errors, respectively, while the vehicle-exposed animals made 1.0 ± 0.5 errors ($p < 0.05$ and $p < 0.01$; Fig. 3B). While all of the vehicle-exposed animals passed the test, 13 - 27 % of the opioid-exposed rats failed to reach the criterion within 20 trials during acquisition and retention testing (Fig. 3C) Statistical analyses (two-way ANOVA) did not reveal any sex differences or any interaction between treatment and sex.

3.4. Spatial learning and memory

Prenatal exposure to methadone or buprenorphine did not affect spatial learning in young adult rats, as shown by equal travel distances and escape latencies for all the three treatment groups during

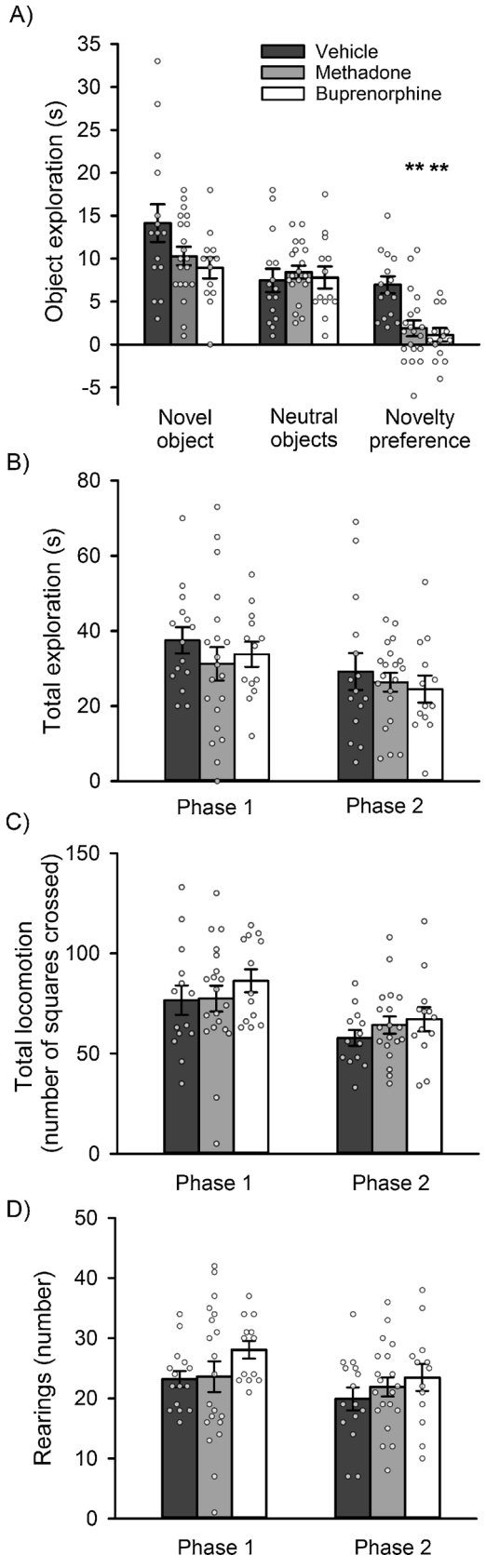

Fig. 2. Maternal exposure to methadone (10 mg/kg/day) or buprenorphine (1 mg/kg/day) during gestation resulted in reduced recognition memory in young adult rat offspring, examined in the novel object recognition test. (A) Time (s) spent exploring the novel object and the neural objects in phase 2. Novelty preference was scored as the difference in time exploring the novel object versus the mean time exploring the neutral objects. (B) Total exploration time (s), (C) total locomotion (number of squares crossed), and (D) number of rearings in phase 1 and 2. The results are shown as mean ± SEM and individual data points. Vehicle $n = 15$ (9 males and 6 females from 8 litters); methadone $n = 20$ (10 males and 10 females from 10 litters); buprenorphine $n = 13$ (8 males and 5 females from 7 litters). $^{**}p < 0.01$ compared to vehicle, two-way ANOVA followed by Dunnett's post hoc test.

acquisition in the MWM test (Fig. 4A and B). Thus, no significant differences were found in the swimming velocity (Fig. 4C). The retention test on day 5 did not reveal any differences among the treatment groups, and the methadone-, buprenorphine- and vehicle-exposed rats spent 15.7 ± 1.3, 15.1 ± 1.2 and 13.3 ± 1.0 s in the NE quadrant, respectively (Fig. 4D). The retention test on day 12, however, revealed a significant effect of the treatment [$F_{(2,52)} = 4.56$, $p = 0.02$]. The methadone-exposed offspring spent 8.3 ± 0.9 s in the NE quadrant, which was significantly less than the 11.2 ± 0.9 s spent by the vehicle-exposed animals ($p < 0.05$; Fig. 4D). No effect was observed for the buprenorphine-exposed rats (12.2 ± 0.8 s; Fig. 4D). Statistical analyses (two-way ANOVA) did not reveal any differences between male and female rats, expect for a shorter swimming distance for the males on day 2 [$F_{(1,52)} = 5.43$, $p = 0.03$]. No significant effect was observed for the interaction between treatment and sex. The visual platform test performed on day 12 did not reveal any significant differences in escape latency among the three treatment groups (data not shown).

4. Discussion

To date, there is great uncertainty related to the use of OMT during pregnancy and possible negative consequences for the child. While neonatal outcomes and short-term effects have been relatively well described, studies examining adolescents or adults are still missing. In the present study, we demonstrate by use of a rat model and three different behavioral tests that continuous maternal exposure to methadone or buprenorphine during gestation impairs cognitive functioning in their young adult offspring. These findings indicate that exposure to methadone or buprenorphine during fetal development induces long-lasting neurobiological changes in the brain.

Young adult rats prenatally exposed to methadone or buprenorphine exhibited impaired recognition memory, shown by reduced novelty preference. Since rats have an innate tendency to explore changes in their environment, the reduced novelty preference in the opioid-exposed offspring may either reflect an impaired ability to notice the novel object or that the animals ascribe less value to the change in the environment. In agreement with our findings, Chen et al. (2015) found reduced recognition memory in rat offspring prenatally exposed to daily injections of methadone or buprenorphine. In several human studies, it has been reported that young children born to mothers in OMT display reduced sustained and selective attention compared to nonexposed children (Konijnenberg et al., 2018; Konijnenberg and Melinder, 2015b; Levine and Woodward, 2018; Wilson et al., 1981); however, it is not known whether this deficit persists into adulthood.

Impaired nonspatial reference learning and memory were observed in both methadone- and buprenorphine-exposed offspring. To our knowledge, we are the first to report this after prenatal exposure to buprenorphine. The opioid-exposed animals took twice as many trials to meet the criterion in the SBD test compared to the control animals during both acquisition and retention testing, which is in fact an underestimation since several of the opioid-exposed animals did not successfully complete the test within the maximum of 20 trials. In accordance with our findings, several studies have reported impaired nonspatial learning and memory in young adult and adult rats exposed to methadone during gestation (Jantzie et al., 2020; Peters, 1977; Van Wagoner et al., 1980; Zagon et al., 1979), while one study shows

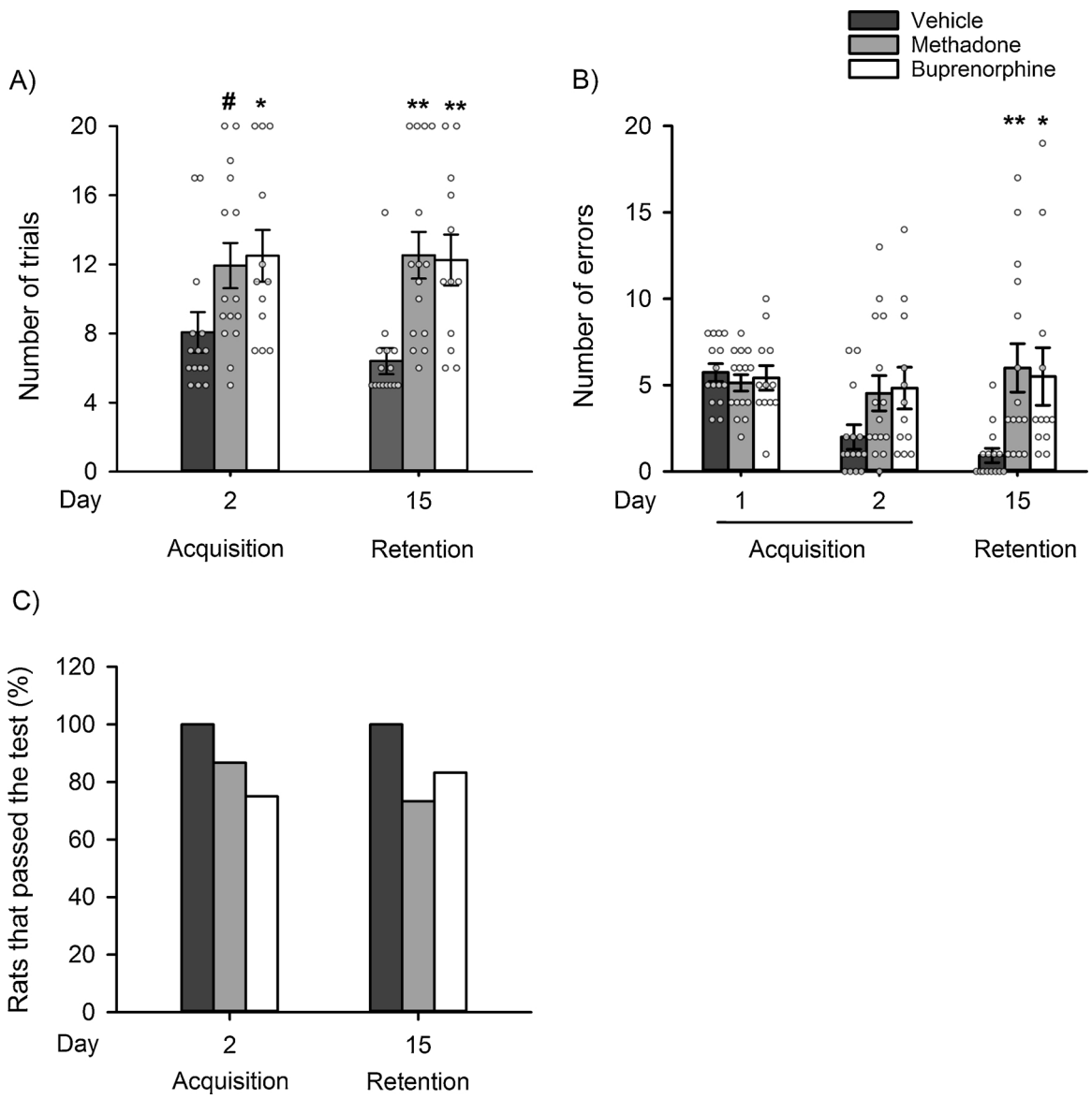

Fig. 3. Maternal exposure to methadone (10 mg/kg/day) or buprenorphine (1 mg/kg/day) during gestation impaired nonspatial learning and memory in young adult rat offspring, examined in the simultaneous brightness discrimination (SBD) test. (A) Number of trials used to reach the criterion of 5 correct responses in succession during acquisition (day 2) and retention (day 15). (B) Number of errors made during acquisition (day 1 and 2) and retention (day 15). (C) Offspring that reached the criterion within the limit of 20 trials (in %) during acquisition (day 2) and retention (day 15). The results are shown as mean ± SEM and individual data points (A and B) and as mean values (C). Vehicle $n = 15$ (9 males and 6 females from 8 litters); methadone $n = 15$ (8 males and 7 females from 10 litters); buprenorphine $n = 12$ (6 males and 6 females from 7 litters). $^{\#}p = 0.06$, compared to vehicle, $^{*}p < 0.05$, $^{**}p < 0.01$, compared to vehicle, two-way ANOVA followed by Dunnett's post hoc test.

normal cognitive functioning (Hutchings et al., 1979).

Prenatal exposure to methadone also resulted in reduced spatial memory. This effect was revealed when the methadone-exposed animals were tested one week after acquisition, suggesting an impaired long-term memory. Testing performed one day after acquisition did not reveal any group differences, indicating preserved short-term memory. This latter finding is in agreement with the work of Chiang et al. (2015); however, in their study the lack of effect one day after acquisition was interpreted as unaffected spatial memory, whereas long-term memory was not examined. We did not observe any effect on spatial memory in the buprenorphine-exposed animals. Consistent with previous findings (Chiang et al., 2015; Hung et al., 2013), the spatial learning was not affected by prenatal exposure to either methadone or buprenorphine.

The major strength of the present study is the extensive examination of cognitive functioning by the use of three behavioral tests examining different aspects of cognition. Another strength is the use of osmotic minipumps implanted prior to mating, ensuring stable maternal blood opioid concentrations that closely resemble those reported in pregnant women in OMT (Bartu et al., 2012; Concheiro et al., 2010; Gordon et al., 2010; Kongstorp et al., 2019). In contrast, daily injections to methadone or buprenorphine during gestation cause narrow and high peak blood concentrations resulting in repeated opioid withdrawal which may influence the study outcomes (Lichtblau and Sparber, 1981). In the present study, several control measures, including the total exploration time, locomotor activity and the number of rearings in the novel object recognition test, as well as the swimming velocity in the MWM test, did not differ between the treatment groups. This strengthens the conclusion that the reduced performance of the methadone- and buprenorphine-exposed animals was caused by cognitive impairments and not by differences in motivation or physical functioning. In clinical studies, it has been reported that children born to mothers in OMT have vision problems (Konijnenberg and Melinder,

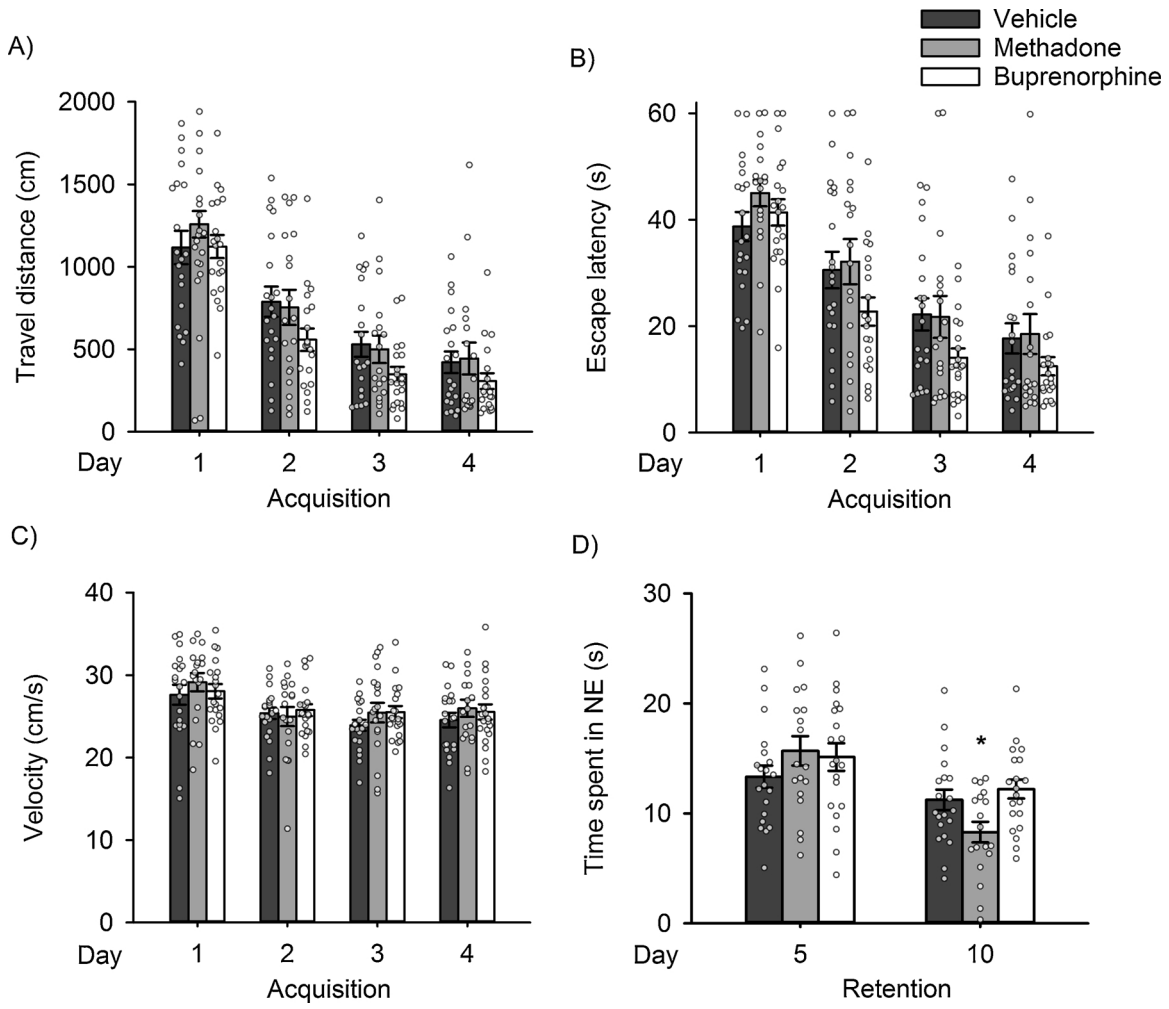

Fig. 4. Maternal exposure to methadone (10 mg/kg/day), but not buprenorphine (1 mg/kg/day) during gestation impaired long-term spatial memory in young adult rat offspring, when examined in the Morris water maze (MWM) test. (A) Travel distance (cm), (B) escape latency (s) and (C) swimming velocity (cm/s) during acquisition (day 1-4). (D) Time (s) spent in the north-east (NE) quadrant during retention testing on day 5 and 12. The results are shown as mean ± SEM and individual data points. Vehicle $n = 20$ (12 males and 8 females from 10 litters); methadone $n = 18$ (9 males and 9 females from 10 litters); buprenorphine $n = 20$ (11 males and 9 females from 10 litters).*$p < 0.05$ compared to vehicle, two-way ANOVA followed by Dunnett's post hoc test.

2013; Melinder et al., 2013). The visual platform test performed in the MWM did not reveal any differences among the treatment groups, suggesting that the impaired performance was not due to visual disturbances.

Concerning neurodevelopment, the newborn rat pup is considered to be comparable to a human fetus at the late second to early third trimester (Semple et al., 2013). Since the methadone and buprenorphine exposure ceased around the time of delivery, the exposure regimen in the present study accounts for the first and second trimester of a human pregnancy. Termination of opioid exposure is associated with withdrawal effects, which have been suggested to be a potential cause for the long-term negative effects reported in offspring following prenatal opioid exposure (Lichtblau and Sparber, 1982). Recently, we reported that offspring exposed to methadone or buprenorphine using the same exposure regimen as in the present study still had opioids present in their brains one week after birth (Kongstorp et al., 2019). The slow elimination of opioids from the offspring´s brain implies that the offspring is gradually weaned off methadone and buprenorphine, which is in accordance with the finding of no change in spontaneous or naltrexone precipitated ultrasonic vocalizations in the methadone and buprenorphine-exposed pups (Kongstorp et al., 2019). However, we cannot completely exclude that the opioid-exposed offspring experienced any minor abstinences.

Reduced maternal caregiving has been suggested as a potential cause for long-term negative effects in children exposed to OMT during pregnancy (Sarfi et al., 2013). We found no differences in the maternal behavior between the three treatment groups when measured 5 days after birth. Moreover, daily monitoring of the dams and litters the first week after birth did not reveal any apparent signs of reduced maternal care or increased number of neonatal deaths. In contrast to our observations, a recent study reported clear signs of maternal neglect in buprenorphine exposed dams; however, these dams were given daily injections continuing until the end of postpartum at PND 21 (Wallin et al., 2019). We previously reported a small decrease in birth weights after prenatal exposure to methadone (Kongstorp et al., 2019); however, this effect was only apparent when comparing individuals ($n = 99$–119) instead of litters ($n = 10$). In the present work, the offspring growth, measured from PND 3–21, was not affected by the treatment, demonstrating that the methadone-exposed pups seem to "catch up" with the vehicle- and buprenorphine-exposed offspring shortly after birth. Taken together, our findings suggest that the impaired cognitive functioning observed most likely is a result of the prenatal methadone or buprenorphine exposure rather than neonatal abstinences or reduced maternal caregiving.

The findings in clinical studies examining long-term outcomes in children exposed to OMT and/or other opioids during fetal life have

been summarized in three recent meta-analyses, which all indicate an increased risk of neurodevelopmental impairments and reduced cognitive performance (Baldacchino et al., 2015; Lee et al., 2020; Monnelly et al., 2019). Because of the more favorable birth outcomes compared with methadone (Jones et al., 2010; Meyer et al., 2015; Welle-Strand et al., 2013; Zedler et al., 2016), buprenorphine is often recommended before methadone during pregnancy; however, the long-term effects of buprenorphine have been less studied. A clinical study reported that preschool children exposed to methadone or buprenorphine during fetal life showed similar cognitive impairments (Konijnenberg and Melinder, 2015a). In accordance with this, we found that prenatal exposure to buprenorphine caused cognitive impairments lasting into adulthood in the same extent as for methadone; however, decreased spatial long-term memory was only observed in the methadone exposed offspring.

The impairments in cognitive functioning observed in experimental animal studies by us and others (Chen et al., 2015; Jantzie et al., 2020; Peters, 1977; Van Wagoner et al., 1980; Zagon et al., 1979) indicate that exposure to methadone or buprenorphine during early development causes long-lasting neurobiological changes in the offspring brain. Spatial and nonspatial learning and memory as well as recognition memory are hippocampal-dependent functions (Clark et al., 2001; Cohen et al., 2013; Morris et al., 1982). A number of cellular alterations have been reported within the hippocampus of offspring prenatally exposed to opioids, including changes in long-term potentiation, long-term depression, synaptogenesis and levels of nerve growth factors (Ahmadalipour et al., 2015; Nasiraei-Moghadam et al., 2013; Schrott et al., 2008; Tan et al., 2015). However, the literature in this field is limited and further studies are needed to pinpoint the underlying neurobiological mechanisms of cognitive impairments induced by prenatal exposure to methadone or buprenorphine.

5. Conclusion

This study shows by use of three different behavioral tests, that prenatal exposure to either methadone or buprenorphine impairs cognitive functioning in young adult rats. Both the recognition memory and the nonspatial learning and memory were reduced compared with the vehicle-treated group. For the methadone-exposed offspring, long-term spatial memory was also negatively affected. Since the opioid exposed dams displayed normal maternal behavior and our recent results showed only minor offspring withdrawal effects, we conclude that the observed impairments in cognitive functioning are more likely a result of the prenatal methadone or buprenorphine exposure rather than an effect of neonatal abstinences or reduced maternal caregiving. Thus, our study points to a negative impact of methadone or buprenorphine on neurobiological development and emphasizes the need for clinical studies to examine long-term cognitive functioning in children born to mothers in OMT.

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Contributors

Participated in research design: Kongstorp, Bogen, Stiris and Andersen.

Conducted the experiments: Kongstorp, Andersen.

Performed data analysis: Kongstorp.

Wrote or contributed to the writing of the manuscript: Kongstorp, Bogen, Stiris and Andersen.

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Declaration of Competing Interest

None.

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