

Primary investigation on the conditioned place preference to parents and cocaine in pre-weanling mandarin vole pups

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Abstract: Social interaction is a substantial natural reward. Similar to drug-induced conditioned place preference (CPP), social reward-CPP is established via a Pavlovian association between the environment and the rewarding effects of social interaction. Socially monogamous mandarin voles (*Microtus mandarinus*) engage in a suite of close social interactions that include parent-offspring bonding; like females, males also provide high levels of parental care to pre-weanling pups. The reinforcing property of pups in parents is known. However, it remains unclear whether parents could become reinforcing to pre-weanling pups. To evaluate the reward associations that pre-weanling pups formed with their parents, the CPP response to dams, sires and a pharmacologic reinforcer (cocaine, 20 mg/kg) was assessed in postnatal day (PND) 13 - 17 and PND 19 - 23 mandarin vole pups. We found that the time that the pups spent in the reinforcing agent-paired chamber and opposite chamber of the apparatus was not statistically different whether conditioned to dams, sires or cocaine respectively. These results indicate that pre-weanling mandarin vole pups failed to form place preferences to their fathers, mothers, or cocaine (at the doses tested).

Key words: Cocaine; Conditioned place preference; Mandarin vole; Social reward

断乳前棕色田鼠幼仔对双亲和可卡因的条件位置偏爱

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摘要: 个体间的社会互动是一种天然奖赏, 这种社会性奖赏诱导的条件位置偏爱 (Conditioned place preference, CPP) 是通过环境信息和社会互动奖赏效应间建立条件反射形成的, 与药物奖赏诱导的 CPP 相似。棕色田鼠 (*Microtus mandarinus*) 是一种社会性单配制田鼠, 具有紧密的亲-子联系和社会互动; 雄鼠对断乳前幼仔也提供较高水平的亲本抚育。幼仔强化能够诱导母鼠及父鼠形成 CPP, 但双亲对幼仔是否具有强化效应还不清楚。为探讨断乳前幼仔与双亲形成的奖赏联系, 本实验检测了出生后 13 - 17 d 和 19 - 23 d 两个发育龄段的棕色田鼠幼仔对母鼠、父鼠以及可卡因 (20 mg/kg) 的 CPP 反应。数据显示在分别用母鼠、父鼠或可卡因强化后, 两个年龄段的幼仔在 CPP 箱的强化室与非强化室所处时间没有显著性差异。这些结果表明断乳前棕色田鼠幼仔不能形成对母鼠、父鼠及可卡因的位置偏爱。

关键词: 棕色田鼠; 社会性奖赏; 可卡因; 条件位置偏爱

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1 Introduction

Maternal care is critical to offspring survival in all mammalian species. Males also engage in parental care in some monogamous species (Dewsbury, 1985; Smorkatcheva, 1999). Monogamous rodents engage in a

suite of complex social interactions including pair bonding and pre-weaning parental care by fathers, thus forming closer social ties with their offspring than do polygamous males (Young *et al.*, 1998; Campbell *et al.*, 2009); the importance of the father is not primarily protection against infanticidal intruders, but rather

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the direct care of young (e. g. providing warmth, grooming). Thus, paternal care in monogamous rodents is often crucial, or at least beneficial, to offspring development and survival (Wang and Novak, 1992; Gubernick *et al.*, 2000; Bredy *et al.*, 2004).

The mandarin vole (*Microtus mandarinus*) is a monogamous rodent species (Smorkatcheva, 1999; Tai *et al.*, 2001; Tai and Wang, 2001) that exhibits lasting father-offspring recognition (Wang and Tai, 2012). During the postpartum period, fathers provide almost exclusive care of the offspring, with the exception of nursing. Paternal behaviors include hovering, licking or grooming pups, body contact with pups, and retrieval of pups (Jia *et al.*, 2009; Wu *et al.*, 2011). Our previous studies on mandarin voles have shown that paternal deprivation affects the physical development of the offspring, the engagement in play-fighting (Wang *et al.*, 2011, 2012a), anxiety levels, paternal behaviors (Jia *et al.*, 2009, 2011) and pair bonding in adulthood (Yu *et al.*, 2012).

Like addictive drugs that are commonly abused, positive social interaction is a rewarding or reinforcing stimulus, which can activate the brain's reward circuitries (Aragona *et al.*, 2007; Neumann, 2009). A conditioned place preference (CPP) paradigm is widely used in studying behavioral and neural processes involved in reward and reinforcement (Tzschentke, 1998). Social reward-CPP is established via a Pavlovian association between the environment and rewarding effects of social interaction, similar to drug-CPP (Thiel *et al.*, 2008). In rats, the rewarding value of pup stimuli is dependent upon full interaction between the mother and her pups (Fleming *et al.*, 1994a; Mattson *et al.*, 2001). Mandarin vole fathers, like maternal rats (Mattson *et al.*, 2001; Wansaw *et al.*, 2008), can form pup-induced CPP throughout the entire postpartum period, indicating that pups are a strong reinforcing agent or rewarding stimuli to their fathers (Wang *et al.*, 2012b). However, it remains unclear whether the reinforcing properties of fathers or mothers are also available in pre-weanling pups.

Bolanos *et al.* (1996) posited that reward processes are functionally mature in pre-weanling rats (at least by 10 days of age). It has been demonstrated that cocaine-or morphine-induced CPP occurs in pre-weanling mice or rats (Laviola *et al.*, 1992; Randall *et al.*, 1998). pre-weanling mandarin vole pups can re-

cognize their fathers or parental odor (Wang *et al.*, 2007; Wang *et al.*, 2012c). Thus, the reward associations pre-weanling pups formed with their parents deserve investigating. In consideration of the influence of the decoration cues of the CPP chamber and reinforcing stimuli on pup preference, we examined the CPP response to dams, sires and a pharmacologic reinforcer (cocaine) in pre-weanling mandarin vole pups using two sets of CPP apparatuses.

2 Materials and methods

2.1 Subjects

Mandarin voles used in this study were laboratory-reared F3 generation animals that originated from a wild population in the Henan province, China. Animals were reared in conventional plastic cages (length \times width \times height, 44 cm \times 22 cm \times 16 cm). Cotton wool and wood shavings were provided for nesting material and were changed weekly. The colony room was illuminated on a 14:10 light-dark cycle (lights on 20:00), and the temperature was maintained at $23^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Water, carrots, and standard rabbit chow (Xi'an Jiaotong University Laboratory Animal Center, Xi'an, China) were freely available. At 70–80 days of age, virgin females and males were paired. Twenty days after pairing, females were checked daily for signs of labor. The day of birth was denoted as postnatal day (PND) zero (day of birth = day 0). Mandarin voles were weaned at roughly 21–23 days. At approximately PND 13, pup eyes open and they become more active outside the nest, after which the fathers engage in active pup retrieval, huddling, and grooming (Smorkatcheva, 2003; Yu *et al.*, 2011). Given that the degree of pup preference for parents may vary with age, PND 13–17 and 19–23 male pups were selected for study. Animal care followed guidelines set by the National Institutes of Health and was approved by the Shaanxi Normal University Institutional Animal Care and Use Committee.

2.2 Experiment 1: Testing by CPP apparatus (a)

The place preference apparatus used in this experiment was the same as described in a previous experiments (Wang *et al.*, 2012b, 2012c). The setup consisted of three equal-sized plexiglas chambers (l \times w \times h, 25 cm \times 20 cm \times 30 cm). The three chambers were separated by two walls each containing a 3.5 cm circular opening so that the pups could access all chambers.

These openings could be closed off with removable dividers during the conditioning phase. The walls of the two outer chambers were covered with either vertical or horizontal alternating 2 cm black and white strips. The walls of the center chamber were covered with gray paper which served as a neutral control, as no cue-stimulus conditioning or exposure took place in this chamber. The floors of the two outer chambers were either perforated or smooth. All contextual cues were counter balanced in a random manner. The position of the apparatus and lighting in the room were kept constant to minimize variability in uncontrolled cues that may have been used as additional conditioning cues by voles. Place preference data were tabulated as time spent in each chamber during the testing.

Pre-test: The objective of the pre-test was to determine whether there was an inherent preference for one of the three chambers prior to conditioning. PND 18 pups ($n = 12$) were used in the pre-test. Pups were initially exposed to each cue-decorated chamber for a 15 min test and allowed to explore for 10 min to acclimate. The apparatus was cleaned with 70% ethanol and allowed to dry between tests. The time spent in each chamber was recorded by a digital video camera and later scored by an experimentally blind rater using Noldus Observe 9.0 (Noldus Information Technology, Wageningen, Netherlands). Additionally, locomotor activities were simultaneously obtained by measuring the total frequencies of pups crossing the doors of the chambers. We compared differences in the number of chamber entries between pups and adult males ($n = 17$) to demonstrate the pups' locomotor activities.

Conditioning with mother: Since we detected no preference for any particular chamber during pre-test, conditioning tests were performed independently of the pre-test. A pup was alternately reinforced with his mother or no mother (PND 13 – 17 pups, $n = 12$; PND 19 – 23 pups, $n = 15$, from different litters). Pups underwent four consecutive days of conditioning where they were alternately reinforced with their mothers or no mother for 2 hours in the morning or afternoon. On the fifth day, pups were given a 15 min test without the presence of the mother. Given that short-term social isolation considerably increases social behavior and the salience or reinforcing value (Niesink and Vim, 1982; Fleming *et al.*, 1994b), pups were deprived of mother for 2 hours on every conditioning day and testing day.

Conditioning with father: Another subset of pups (PND 13 – 17 pups, $n = 12$; PND 19 – 23 pups, $n = 15$) was alternately reinforced with a father or no father. The CPP procedure was identical that described for mother conditioning (Wang *et al.*, 2012c).

2.3 Experiment 2: Testing by CPP apparatus (b)

This apparatus (b) was same to that described by Wang *et al.* (2012d). Mandarin voles get acclimated to a fossorial mode of life (Smorkatcheva, 1999; Tai and Wang, 2001). Since pre-weanling pups live in a burrow before transition from the natal nest to the ground, considering the pups' probable poor vision, in this apparatus (b), the differences in visual cues between the chambers are designed more obviously compared to the first CPP apparatus (a). Briefly, it consisted of two larger chambers ($1 \times w \times h$, 34 cm \times 25 cm \times 32 cm) separated by a smaller middle compartment ($1 \times w \times h$, 11 cm \times 25 cm \times 32 cm). One chamber had gray walls (gray chamber). The other chamber had white-black striped walls (striped chamber). The middle chamber was the acclimation chamber with a 7 cm \times 9 cm door in the center of the base.

Subjects: We selected a new subset of PND 19 – 23 pups.

Pre-test: The pre-test was used to determine whether there was an inherent preference for one of two larger chambers before conditioning. PND 18 pups ($n = 8$) were used in the pre-test. The frequencies of pups crossing the doors of chambers were recorded. We also compared differences in the number of chamber entries between pups and adult males ($n = 12$).

Conditioning with father: We detected no preference for two lateral chambers during pre-test. The conditioning procedure was identical to experiment 1 ($n = 6$).

Conditioning with cocaine: Cocaine-hydrochloride (Northwest Pharmaceutical Co., Ltd. Sinopharm, Xi'an, China) was diluted in saline (0.9% NaCl). Pups ($n = 8$) were conditioned using both cocaine (20 mg/kg) and saline. Both cocaine and an equivalent volume of physiological saline injections were given on the same day for four consecutive days. Specifically, in the morning, subjects were placed in one of the outer chambers with cocaine injections and were placed in the opposite chamber with saline injections in the afternoon. Two injections were administered per day in an alternating counter balanced sequence for

four days to provide four associative pairings for cocaine and saline. Pups were conditioned for 2 hours after injections. The morning session and the afternoon session were at least 6 hours apart, allowing time for cocaine clearance (Thiel *et al.*, 2008). Cocaine doses were chosen on the basis of previous studies on the expression of conditioned preferences in mandarin voles and pre-weanling rats (Bolanos *et al.*, 1996; Wang *et al.*, 2012d).

3 Results

3.1 Experiment 1

The time that the pups spent in each chamber of the apparatus showed no statistical differences during the pre-test ($F_{2,33} = 0.631$, $P > 0.05$) (Fig. 1).

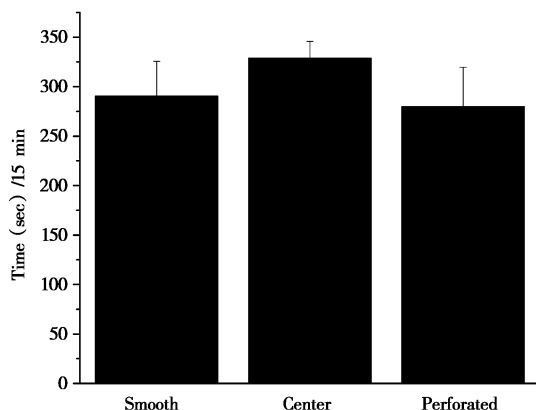


Fig. 1 The time spent by PND 18 mandarin vole pups in the three chambered apparatus (apparatus a) during pre-test. Data are presented as Mean \pm SE ($P > 0.05$)

Following conditioning with mother or father, neither PND13 – 17 nor PND 19 – 23 pups exhibited any preference for their mothers (PND13 – 17: $F_{2,24} = 1.334$, $P > 0.05$; PND19 – 23: $F_{2,24} = 0.341$, $P > 0.05$) or fathers (PND13 – 17: $F_{2,33} = 0.958$, $P > 0.05$; PND19 – 23: $F_{2,42} = 0.063$, $P > 0.05$) cue-associated chambers (Fig. 2).

3.2 Experiment 2

The pre-test results showed that all pups spent almost an equal amount of time in the two lateral compartments ($t = 0.205$, $P > 0.05$) (Fig. 3). There were no differences between pups and adult males in the number of chamber entries in apparatus (a) ($t = -0.177$, $P = 0.861$) or apparatus (b) ($t = 1.136$, $P = 0.271$) (Fig. 4).

After conditioning with father or cocaine, PND 19 – 23 pups did not show a significant preference for ei-

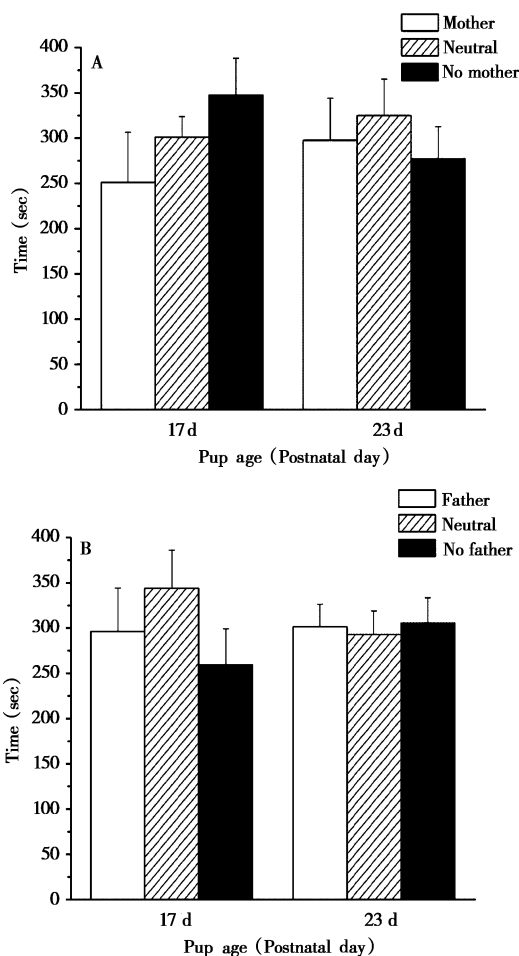


Fig. 2 The time spent by mandarin vole pups of different ages in chambers of apparatus (a) following four days of conditioning with mothers (A) or fathers (B) (see Wang *et al.*, 2012c). Data are presented as Mean \pm SE ($P > 0.05$)

ther stimulus (father: $t = 0.065$, $P > 0.05$; cocaine: $t = 1.679$, $P > 0.05$) (Fig. 5 and 6).

4 Discussion

Our study is the first to use the CPP paradigm to examine the reward associations of pups with parents in a socially monogamous rodent species. The results on paternal reinforcement in experiment 2 agreed with previous findings (Wang *et al.*, 2012c), indicating that the decoration cues of the CPP chamber did not affect the pups' preferences during the CPP test. Although pre-weanling mandarin vole pups can recognize their parents (Wang *et al.*, 2007, 2012c), unexpectedly, pups did not form a preference for father or mother cues, suggesting that the parental reward-CPP cannot be established via Pavlovian conditioning using reinfor-

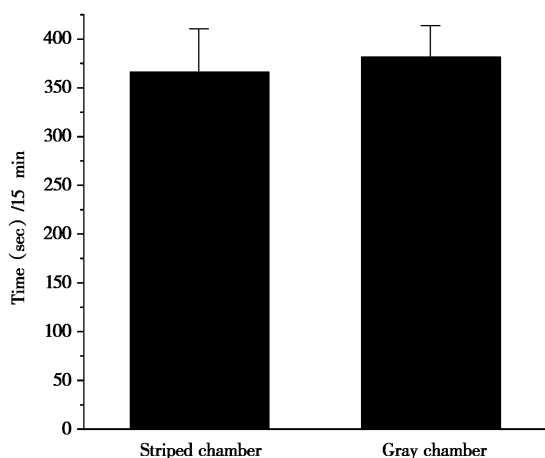


Fig. 3 The time spent by PND 18 mandarin vole pups in two lateral compartments apparatus (b) during pre-test. Data are presented as Mean \pm SE ($P > 0.05$)

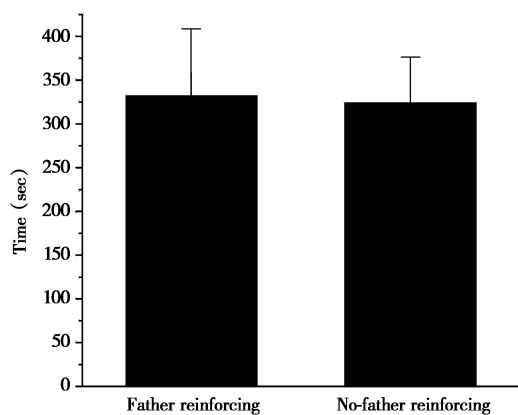


Fig. 5 The time spent by PND 19 – 23 mandarin vole pups in chambers of apparatus (b) following four days of conditioning with fathers. Data are presented as Mean \pm SE ($P > 0.05$)

cing stimuli and environment cues. In contrast, using the same experiment procedure and CPP apparatus, mandarin vole fathers did form a CPP for pups in cue-associated chambers (Wang *et al.*, 2012b). Mattson *et al.* (2001) also found that maternal rats show a preference for pup cue-associated chamber. The number of entries between chambers did not correlate with the duration of time spent in the chamber. Entries instead proved a useful control measure of general activity (Nadler *et al.*, 2004; Aragona *et al.*, 2007). From PND18 on, pups have similar levels of movement compared to adult males in apparatus (a) and apparatus (b). Thus, the ‘negative’ performance of pups during the CPP test was not due to locomotor activity. These results suggest that the rewarding effects of par-

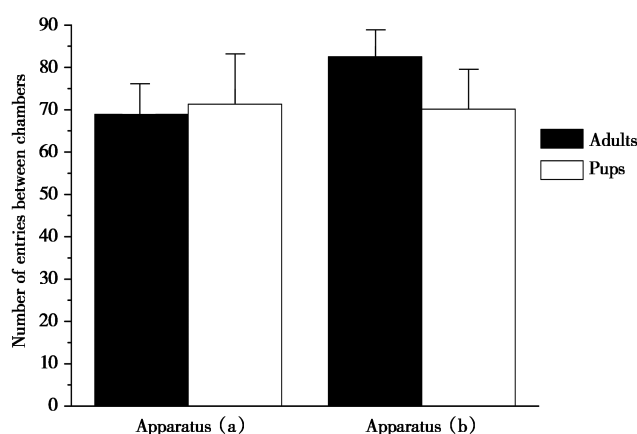


Fig. 4 Number of entries between chambers in mandarin vole adults and PND 18 pups within apparatus (a) and apparatus (b) during pre-test. Data are presented as Mean \pm SE ($P > 0.05$)

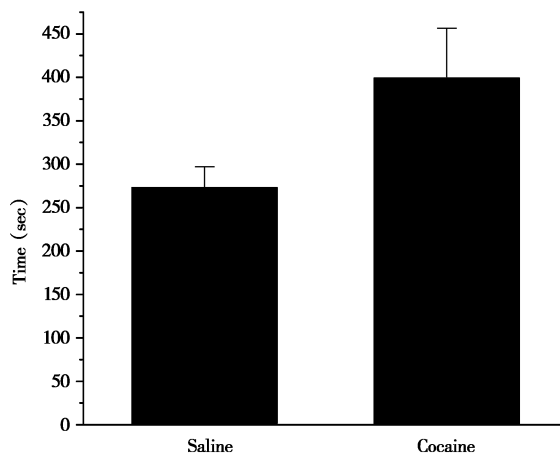


Fig. 6 The time spent by PND 19 – 23 mandarin vole pups in chambers of apparatus (b) following four days of conditioning with injections of saline and 20 mg/kg cocaine. Data are presented as Mean \pm SE ($P > 0.05$)

ents to their pups are not similar to that of pups to their parents.

Another interesting finding is that PND 19 – 23 pups did not exhibit cocaine-induced CPP. Although the pups tended to prefer cocaine cues-associated chamber, the differences were not significant. In contrast, adult male and female mandarin voles readily form cocaine CPP (Wang *et al.*, 2012b, 2012d). Similarly, a single 25 mg/kg cocaine dose resulted in CPP in 28 – 31 day-old mice, but not in 14 – 17 and 21 – 24 day-old mice (Dell’omo *et al.*, 1993). Likewise, morphine-induced CPP could be established in adult mice but not in adolescent (28 – day-old) mice (Qi *et al.*, 2011). A possible explanation for these

findings may be related to a difference in sensitivity to the pharmacological effects of the drug between the adults and their young.

Similar to our experimental procedure, Bolanos *et al.* (1996) found that morphine was consistently able to induce CPP in two pre-weanling age groups (10 – and 17 – day-old), but not in peri-adolescent (35 – day-old) rats. However, using the same procedure, a different group of similarly aged rats showed conditioned preference produced by 20 mg/kg cocaine. At the 25 mg/kg cocaine dose, CD1 mice pups developed CPP at three different developmental ages (14 – 17, 21 – 24, or 28 – 31 days), while the 5 mg/kg dose was effective only in 21 – 24 day pups, and the 1 mg/kg dose was ineffective (Laviola *et al.*, 1992). These results show that pre-weanling pups have different sensitivities to different drugs. Cocaine and morphine in place preference paradigms are mediated by different neural systems. This difference may be related to the different mechanisms of action of these two compounds (Belzung and Barreau, 2000). We also cannot eliminate the possibility that the response to the drug in pre-weanling pups is species-specific and thus highly variable across species. There are differential effects in different species, animal ages, and treatments. Additionally, the cocaine dose could also affect CPP establishment. More comprehensive dose response experiments are needed to determine the wider impact of drug on CPP.

The CPP task involves learning processes (Fleming *et al.*, 1994b; Randall *et al.*, 1998). Social reward-CPP is established via Pavlovian associations between the environment and the rewarding effects of social interaction, similar to that of drug-induced CPP (Thiel *et al.*, 2008). The process of drug addiction shares striking commonalities with the neural plasticity associated with natural reward learning and memory (Kelley, 2004). The reinforcing stimuli may influence learning and memory processes resulting in long-term memory of the reinforcer. The capability to learn through reinforcing stimuli motivated spatial discrimination appears related to the context in which the training occurs (Smith and Croft, 1985). Since body touch (taction) and tickling can induce reward associations in adolescent rodents (Burgdorf and Panksepp, 2001; Kummer *et al.*, 2011), pre-weanling mandarin vole pups may experience the rewarding effects of mothering

and fathering, but they cannot learn from this conditioning experience (environment cues associated with the reinforcing agent) or maintain such a memory. The development of ordinary memory and addictive memory to environments in pups is quite different from adults (Qi *et al.*, 2011). These uncertainties warrant further evaluation. Despite the lack of CPP associations in this study, we believe that the socially monogamous species are good model for studying the attachment rewards of fathers and mothers in pups and the reciprocal interactions of sociality and drug abuse.

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