Neurobiology of Learning and Memory 141 (2017) 78–83

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

journal homepage: www.elsevier.com/locate/ynlme

Dopamine dependent setting of a circadian oscillator underlying the memory for time of day

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ARTICLE INFO

Article history:
Received 11 December 2016
Revised 17 March 2017
Accepted 23 March 2017
Available online 30 March 2017

Keywords:
Amphetamine
Haloperidol
DA
Time-of-day learning
Memory
Place preference
Circadian rhythms

ABSTRACT

Animals learn and remember the time of day that significant conditions occur, and anticipate recurrence at 24-h intervals, even after only one exposure to the condition. On several place-conditioning tasks, animals show context avoidance or preference only near the time of day of the experience. The memory for time of day is registered by a circadian oscillator that is set at the time of the training. We show that manipulations of dopamine (DA) neurotransmission can set a time memory in place preference and avoidance tasks, indicating that time of day is part of the context that is learned. Single injections of the DA agonist, d-amphetamine sulfate given without further exposure to the conditioning apparatus, can reset the timing of anticipatory behavior evoked by previously acquired place-event associations. The data support a model for time memory in which DA signaling sets the phase of a circadian oscillator, which returns to the same state at regular 24-h intervals. The data also raise the possibility that some apparent impairments of memory formation or retention could reflect post-experience resetting of the optimal retrieval time rather than impairment of memory or retrieval *per se*.

1. Introduction

Daily patterns of spontaneous locomotor behavior in mammals are orchestrated by a hierarchical system of circadian clocks. The temporal program of physiology and behavior is coordinated by a central biological clock in the hypothalamic SCN (Moore & Eichler, 1972; Ralph, Foster, Davis, & Menaker, 1990; Stephan & Zucker, 1972). One of the primary functions of the SCN clock is to synchronize the circadian hierarchy with the regular 24-h cycles of the natural environment, enabling a mechanism whereby regularly occurring conditions can be anticipated. However, important conditions in the environment may occur at unexpected times of day, and often without warning. So, for animals to anticipate important conditions, they must make an assessment of the overall likelihood of whether and when the conditions will occur. For animals, the short term adaptations to temporally limited conditions or events includes the learning and memory of time of day as a context feature. It is exhibited in the expression of FAA (Mistlberger, 1994), and in a variety of TPL (Mulder, Gerkema, & Van der Zee, 2013) and place-conditioning tasks (Ralph et al., 2002).

Several lines of evidence indicate that memory for time of day requires the action of circadian oscillators, FEOs and/or CEOs, that are distinct from the SCN clock (Mistlberger, 1994, 2011; Mulder, Gerkema, et al., 2013; Ralph et al., 2013; Stephan, 2002). In most TPL and food anticipation experiments, time of day is presented as a discriminative cue. In these situations, animals learn *explicitly* that the unconditioned stimulus (e.g. food or mild foot shock) is going to occur at one time but not at another. However, animals also exhibit anticipation of significant conditions after experiences at only one time of day, and even after single trials, suggesting that the recurrence of the condition after 24 h is expected. This is *implicit* time memory, where an animal's ability to remember the time of day is inferred from its subsequent anticipatory behaviors. It has been demonstrated in numerous animal species from insects (Beling, 1929; Pahl, Zhu, Pix, Tautz, & Zhang, 2007) to mammals (Cain, Chou, & Ralph, 2004; Holloway & Wansley, 1973a, 1973b; Kamin, 1957; Ralph et al., 2002; Wansley & Holloway, 1975), including primates (Valentinuzzi et al., 2008).

Neither the SCN nor the canonical molecular clock underlying circadian rhythm generation is required for the acquisition or expression of implicit time memory. Behaviorally arrhythmic hamsters with lesions of the SCN continue to demonstrate spontaneous 24-h rhythms of peak performance following conditioning with

Abbreviations: CEO, condition entrainable oscillator; CPA, conditioned place avoidance; CPP, conditioned place preference; FAA, food anticipatory activity; FEO, food entrainable oscillator; SCN, suprachiasmatic nucleus; TPL, time-place learning; ZT, Zeitgeber time.

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http://dx.doi.org/10.1016/j.nlm.2017.03.015
1074-7427/

several learning and conditioning paradigms (e.g. *passive avoidance*, Cain, Chalmers, & Ralph, 2012; *CPP*, Ko, McDonald, & Ralph, 2003; *CPA*, Cain, McDonald, & Ralph, 2008; Cain & Ralph, 2009; *amphetamine sensitization*, Cain, Featherstone, & Ralph, 2011). In mammals, implicit time memory is established by the setting of a circadian oscillator at the time of learning. The clock may be re-entrained subsequently by the SCN without the loss of memory for other context features (Ralph et al., 2013). Furthermore, 24-h oscillations in CPA responsiveness continue in hamsters expressing the short (20-h) circadian period mutation, $Ck1\varepsilon^{tau/tau}$; and conditioning the same strain at 20 h intervals also fails to eliminate 24-h oscillations following training (Cain, Yoon, Shrestha, & Ralph, 2014). Therefore, the acquisition of implicit time memory does not require the canonical molecular clock mechanisms, nor is it classically conditioned.

Extra-SCN control of behavioral rhythms is not itself a novel concept. Both FAA (Mistlberger, 2009, 2011) and 24-h TPL (Mulder, Van Der Zee, Hut, & Gerkema, 2013; Takasu et al., 2012) persist in the absence of a functional SCN or molecular clock. However, the neural substrates responsible for generating these circadian oscillations are poorly understood. A diverse literature points to the involvement of the ascending DA reward pathways in food entrainment and time memory. DA activity is essential to the positive reinforcement of behavior (Wise, 2004), and the establishment of place and context memory (Lukoyanov, Pereira, Mesquita, & Andrade, 2002). It plays an important role in the direct response to aversive stimuli as indicated by increased DA release (Abercrombie, Keefe, DiFrischia, & Zigmond, 1989) and metabolism (Fadda, Melis, & Argiolas, 1978; Lavielle et al., 1979; Robinson, Becker, Young, Akil, & Castaneda, 1987; Shanks, Zalcman, Zacharko, & Anisman, 1991; Thierry, Tassin, Blanc, & Glowinski, 1976). It is also responsive to conditioned stimuli (Deutch, Tam, & Roth, 1985; Dunn, 1988; Herman et al., 1982; Robinson, Becker, & Presty, 1982; Thierry, Tassin, Blanc, & Glowinski, 1978; Young, Bronstein, & Akil, 1993).

The dopaminie agonist, d-amphetamine sulfate, is effective in producing CPP in rodents (Carr & White, 1983; Spyraki, Fibiger, & Phillips, 1982). Sensitization to amphetamine is expressed in a circadian rhythmic pattern, and persists in arrhythmic, SCN lesioned animals (Cain et al., 2011). Therefore, because CPP produced by other reward stimuli are expressed in a circadian fashion with peaks near the time of conditioning (Ralph et al., 2002), we hypothesized that DA neurotransmission carries information regarding the timing of conditioning.

To test the hypothesis, we performed four experiments. In the first two, we determined whether amphetamine or the DA antagonist, haloperidol, as the unconditioned stimulus (US) could also condition time memory when used in used in CPP and CPA paradigms, respectively. This particular neuroleptic was chosen as it has the most general DA receptor antagonism, acting on D2, D3 and D4 receptor subtypes, while being largely specific to DA (i.e., little antagonism of other neurotransmitter receptors) (Blin, 1999). In Experiment 3, we asked whether DA activation is sufficient for setting a time memory by determining whether amphetamine could reset a previously established CPA produced by a mild foot shock as the US. In Experiment 4, we determined whether a chronic application of haloperidol during amphetamine conditioning could block the acquisition of a time memory associated with foot shock CPA.

2. Methods

2.1. Animals

Male hamsters (*Mesocricetus auratus*) were obtained from a breeding colony at the University of Toronto Biosciences Support Facility. Animals were 16–22 weeks of age at the start of each experiment. Throughout each experiment, animals were maintained individually in polypropylene cages (23 cm × 44 cm × 20 cm) with food and water available *ad libitum*. Animals were maintained on a light-dark cycle consisting of 14 h of light and 10 h of dark (LD14:10). Each animal had free access to a running wheel (17 cm diameter) permanently mounted in the cage. Wheel running activity was recorded continuously in 6 min bins using VitalView (Phillips-Respironics, Inc., Bend, Oregon). For all animals, entrainment of circadian rhythms to the LD cycle throughout each experiment was confirmed by visual inspection of activity records.

2.2. Procedures

2.2.1. Experiments 1&2: Place-conditioning

In these experiments, one of two previously neutral contexts was paired with either the DA agonist, d-amphetamine sulfate (1.5 mg/kg in 0.2 ml saline, ip.) or the antagonist, haloperidol (0.5 mg/kg in 0.2 ml saline, ip.). All animals were conditioned for 8 days, being alternately exposed to either a drug-paired context or an unpaired context on successive days. Contexts chambers differed in shape, wall pattern, and odor (amyl acetate, *banana*; and eucalyptus). On Day 1, animals were placed one at a time into an alley connecting the two chambers, and were given 20 min. to explore the alley and both chambers to ensure context neutrality. Habituation and conditioning trials were conducted at the same ZT (ZT11) for all animals. Lights off was defined as ZT12 so that context exposures always occurred in the light, following the procedure used by Ralph et al. (2002). For conditioning trials (Days 2–9), individuals were given a single ip. injection of a drug or saline vehicle 10 min. prior to being placed into one of the two chambers with access to the second chamber blocked. Control vehicle solutions for amphetamine and haloperidol were prepared separately, and were tested on independent groups of animals. Conditioning comprised eight 10 min exposures per day, alternating daily between the drug-paired chamber (4 exposures) the vehicle paired chamber (4 exposures). On test day (Day 10), animals were placed in the connecting alley, and allowed free access to both chambers for 20 min. The difference in time spent in each chamber (dwell time) determined the conditioned preference for or avoidance of the paired context. Entry into a chamber was defined as the moment both forepaws were touching the chamber floor. Similarly exit from a chamber was defined as the moment both paws were place on the alley surface. Fifty percent of the groups trained at ZT11 were tested at ZT11 (ON time) and 50% at ZT03 (OFF time). To avoid extinction issues, animals were tested only once.

2.2.2. Experiment 3: Amphetamine induced clock resetting

Based on the outcome of Experiment 1, we examined the effect of amphetamine on a previously established CPA time memory. Procedures were as described above but with two changes. First, a mild foot shock (5 s. at 0.8 mA), applied at 3, 6, and 9 min into the 10-min exposure to the paired context at ZT11. No shocks were delivered in the unpaired context. Mild foot shock was shown previously to condition a robust place avoidance and memory of time of day (Cain et al., 2004; Ralph et al., 2013). Conditioning was performed. Second, a single amphetamine or vehicle injection was given to each animal in its nest box on the day following the final conditioning trial; and the test trial was moved to Day 11. The injections were given at ZT03 on Day 10, and test times were ZT03 and ZT11 on Day 11.

2.2.3. Experiment 4: Chronic haloperidol administration and CPA time memory

Twelve animals received subcutaneous interscapular implants of slow-release haloperidol pellets (Innovative Research of Amer-