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Recruitment of hippocampal neurons to encode behavioral events in the rat: Alterations in cognitive demand and cannabinoid exposure

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Abstract

Successful performance by rats of a delayed-nonmatch-to-sample (DNMS) task is hippocampal dependent. We have shown that neurons in hippocampus differentially encode task-relevant events. These responses are critical for correct DNMS performance and are diminished by exogenous cannabinoids. We therefore reasoned that hippocampal neural correlates of behavior are likely shaped during learning; however, to date, no work has examined these correlates during DNMS acquisition training. Consequently, the present study assessed the emergence of hippocampal neural encoding when (i) cognitive task demands were increased through prolongation of delay intervals between sample and nonmatch phase, and (ii) when animals are under cannabinoid treatment and performance is compromised. Adult, male Long-Evans rats were trained to perform the DNMS task without delay and then implanted with multi-electrode recording arrays directed to CA3 and CA1 sub-fields of the hippocampus. Following recovery, single units were isolated and animals divided into two treatment groups: vehicle or WIN 55,212-2 (WIN-2, 0.35 mg/kg). Ensemble firing was monitored during retraining in DNMS task at 0s, and subsequently delay intervals were progressively increased to 1–10s, 11–20s and 21–30s when animals met criterion (80% correct) at each respective interval. Hippocampal CA3 and CA1 principal cells were isolated and recorded throughout treatment. Extension of the delay led to an increase in the number of task-correlated neurons in controls. This recruitment of novel cells was reduced/prevented in the presence of WIN-2 and was paralleled by impairment in acquisition learning at longer delay intervals. Moreover, WIN-2 suppressed hippocampal ensemble firing during the sample (encoding) but not nonmatch phase of the DNMS task across all delays. These cannabinoid induced alterations in hippocampal neuronal activity may explain the observed deficits in DNMS performance.

Keywords

cannabinoids; hippocampus; ensemble; short-term memory; learning; delayed-non- match-to sample; electrophysiology

Introduction

Studies utilizing the delayed-nonmatch-to-sample (DNMS) task in rodents have revealed four different functional cell types (FCTs) of hippocampal neurons with distinct firing characteristics and correlates to specific task-relevant events (Hampson and Deadwyler, 2000). Single-unit recordings from CA3 and CA1 hippocampal sub-fields during DNMS performance confirm that hippocampal principal cells transiently increase firing rates in the sample (encoding) and nonmatch (recall) phases of the DNMS task and gradually ramp up their discharge frequency during the course of the delay prior to a correct response (Hampson et al., 2003; Hampson and Deadwyler, 2000). The differential encoding of distinct DNMS behavioral events is thus the hallmark of FCTs which are shaped to respond distinctly during sample, delay and nonmatch phases, and comprise an overall ensemble 'code' that is crucial for performing the DNMS task successfully (Hampson and Deadwyler, 2000; Hampson et al., 1999b). When recorded as multi-neuron ensembles, it is possible to decipher the neural code, identify DNMS behavioral events, and predict behavioral performance (Hampson et al. 2007). We have also shown that hippocampal neural coding is influenced by recent experience and the sequence in which behavioral trials are encountered (Deadwyler and Hampson 2006), thus suggesting that the hippocampal encoding of the DNMS task should "develop" during DNMS training. Since hippocampal neural firing patterns are altered in the presence of cannabinoid receptor (CB1) agonists (Hampson and Deadwyler 2000), we further hypothesize that cannabinoid exposure during task acquisition should alter the formation of the hippocampal neural code for DNMS events.

The primary psychoactive constituent of marijuana, Δ^9 -tetrahydrocannabinol (Δ^9 -THC) and synthetic cannabinoid agonists, such as WIN 55212-2 (WIN-2), bind to G-protein coupled CB1 receptors, which are abundantly expressed in the hippocampus, neocortex, cerebellum and basal ganglia (Herkenham et al., 1991; Ameri, 1999; Freund et al., 2003). Numerous studies provide compelling evidence that systemic and intra-hippocampal injections of cannabinoids impair memory in hippocampal-dependent tasks (Fadda et al., 2004; Ferrari et al., 1999; Hampson et al., 2003; Hampson and Deadwyler, 2000; Lichtman et al., 1995; Lichtman and Martin, 1996; Robinson et al., 2007) and confirm that hippocampal neural activity is crucial for performance in both spatial and non-spatial memory tasks which incorporate a brief delay interval (Correll and Scoville, 1965; Olton and Feustle, 1981; Akase et al., 1989; Rawlins et al., 1993; Sweeney et al., 1997; Steele and Morris, 1999). Prior work from this laboratory has demonstrated similarities in the delay-dependent impairment of DNMS performance following administration of cannabinoids such as Δ^9 -THC and WIN-2 (Hampson and Deadwyler, 2000; 1998) compared to selective removal of hippocampus with ibotenate induced lesions (Hampson et al., 1999a).

Documentation of FCTs and their modulation by cannabinoids have, to date, been performed only in fully-trained subjects (i.e. pre-trained to perform the task with 1–30s delay intervals at 80% correct responding); thus it is unclear how these ensemble codes emerge and how they are altered when task demands, for example cognitive load, is increased. Given that cannabinoids can reduce the number of FCTs and suppress hippocampal firing during sample phase and delay intervals (Hampson and Deadwyler, 2000), we also explored how cannabinoid treatment altered acquisition learning and ensemble firing when delay intervals were extended in the DNMS task.

Materials and Methods

Animals

Twelve male, Long-Evans rats (Harlan) aged between 4–6 months were individually housed in a temperature-controlled (20–22°C) environment with a 12-h light/dark cycle (lights on 7

am to 7 pm). All animals were water restricted, but allowed free access to food throughout pre-training and drug testing. The volume of water consumed and weights were monitored daily to maintain 83% of *ad libitum* body weight. Water consumption during the behavioral testing was recorded and a supplemental volume was given immediately after the session to provide 20–22 hours of water deprivation. All animal protocols were approved by the Wake Forest University Institutional Animal Care and Use Committee and were in concordance with the National Institute of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978).

Apparatus

The apparatus has been described previously (Heyser et al., 1993; Deadwyler et al., 1996) and consisted of a 43 × 43 × 50 cm Plexiglass behavioral testing chamber with two retractable levers (left and right) positioned on either side of a water trough in the front panel and a nose-poke device mounted in the center of the back panel. A cue light (28-V) was positioned immediately above the nose-poke device. The test chamber was illuminated by two 28-V incandescent house lights mounted on the top of the chamber. The entire apparatus was computer-controlled and housed inside a sound-attenuated cubicle.

Drug preparation and administration

The potent CB1 receptor agonist, WIN-2, was obtained as mesylate powder (Sigma, MO) and was dissolved in ethanol as a 20 mg/ml stock solution. The detergent vehicle was prepared from Pluronic F68 (Sigma, MO) as a 20 mg/ml solution in ethanol. WIN-2 stock (0.5 ml) was mixed with 2.0 ml of vehicle to which a further 2.0 ml of saline (0.9%) was slowly added. The solution was stirred rapidly and placed under a steady stream of nitrogen gas to evaporate the ethanol for 10 minutes. This resulted in a suspension (5.0 mg/ml of WIN-2, <0.01% ethanol) which was then sonicated and diluted with saline to the final injection dose (0.35 mg/kg). The vehicle (Pluronic F68) solution was prepared in a similar manner without drug. Both drug and vehicle solutions were made fresh everyday.

Behavioral training and testing procedure

Pre-training—All animals were pre-trained to perform the DNMS task without a delay (0s) prior to implantation of multi-electrode arrays. The DNMS task consists of three main phases: sample, delay and nonmatch. At the initiation of a trial, either the left or right lever was extended at 50% overall probability and the animal was required to press this lever (sample response, SR) to complete the sample phase of the task. The lever was immediately retracted, initiating the delay phase, signaled by the illumination of a cue light over the nose-poke device in the rear panel. The subject was required to nose-poke in the photocell device at least once during the delay before the interval was terminated. The last nose poke after the delay timed out turned off the cue light and simultaneously extended both levers in the front panel, signaling the onset of the nonmatch phase, during which the animal had to press the opposite lever to that pressed during the sample phase to be rewarded with a droplet of water, which was also noted as a ‘correct’ response. At the termination of the nonmatch phase, the levers were retracted for a 10s inter-trial interval before starting the next trial. If the subject opted to select the same lever as the sample, this was counted as an ‘error’ response and the animal was not rewarded. Individual sessions consisted of 100 trials per day and the performance was assessed as % correct responses.

Drug testing—Following surgery, animals were administered with a daily dose of either WIN-2 (0.35mg/kg; $n = 6$) or vehicle (Pluronic F68; $n = 6$) approximately 15–20 minutes prior to running them on the DNMS task. Initially, all subjects were required to achieve criterion (80% correct responses) at virtually no delay (0s). Thereafter, all animals were

subjected to perform the task with trials at progressively longer delay intervals ranging from 1–10s; 11–20s and 21–30s respectively, they progressed to the next interval once 80% correct responses per session was met. All subjects were tested during the light phase (i.e. between 10am and 3pm).

Analysis of behavioral data—The total number of training days required to achieve criterion (80% correct responses) at each delay interval (0s; 1–10s; 11–20s and 21–30s) were recorded for both cannabinoid and vehicle treated groups. One-way repeated measures ANOVAs were performed to assess statistical significance across delay intervals for both vehicle and cannabinoid treated groups. Paired *t*-tests were used to assess differences between individual delay intervals.

Surgery—Following pre-training animals were anesthetized under a constant flow of isoflurane (1.5%)/O₂ (100%) mixture and placed in a stereotaxic frame. A craniotomy (5mm-diameter) was performed over the dorsal hippocampus and the center pair of array electrodes consisting of sixteen stainless steel wires (diameter: 40µm each) was positioned at co-ordinates 3.4 mm posterior and 3.0 mm lateral to Bregma (Paxinos and Watson, 1998). The longitudinal axis of the array was angled 30° to the midline, with posterior electrode sites more lateral than anterior sites. The array was driven in 25 – 100 µm steps to a depth of 3.0 – 4.0 mm from the cortical surface for CA3 leads (eight long wires), with the CA1 leads (eight short wires) automatically positioned 1.2 mm higher along the longitudinal axis of the hippocampus. The array was designed such that the distance between two adjacent electrodes within a given row was 200 µm and between rows (long and short wires) was 400 µm apart. Neural activity from the micro-wire electrodes were monitored throughout surgery to ensure placement in appropriate hippocampal cell layers and the exposed cortex was kept moist with 0.9% sodium chloride. After array placement, the cranium was sealed with bone wax and dental cement, and the animal was allowed to recover for at least 1 week (Hampson and Deadwyler 2000).

Multi-neuronal recording—Once cables were connected, neural activity (extracellular action potentials, or ‘spikes’), behavioral events and behavioral responses within each DNMS trial were recorded for each implanted animal. Single neurons recorded from each of the 16 micro-wire electrodes were digitized at 40 kHz and time-stamped along with behavioral events for computer processing within each DNMS session (Hampson et al., 1996, 2000, 2003). Individual neuronal action potentials were isolated by time-amplitude window discrimination and computer-identified individual waveform characteristics using a multi-neuron acquisition (MAP) processor (Plexon Inc., Dallas, TX, USA). Single-units from each of the electrodes were recorded daily using waveform and firing characteristics within the task (peri-event histograms) following respective treatments. Only spike waveforms with associated firing rates consistent with behavioral correlates across sessions were included in the analysis.

Analysis of neuronal data—Hippocampal principal cells identified from selected wires in CA3 and CA1 sub-fields were recorded daily to ensure that: 1) where possible, isolated neurons were conserved from day to day, and 2) cells were distinct and the same cell was not recorded as two or more separate units. Thus, only spike waveforms with associated firing rates and peri-event histograms (i.e., behavioral correlates) that were consistently the same across sessions were included in the analysis to maximize the likelihood that the same neurons were recorded continuously following respective treatments (Hampson et al., 2003; Hampson et al., 1996). The total number of cells isolated on criterion achieving days at each delay was recorded following each respective treatment. In addition, FCTs with distinct firing characteristics during task specific events on both correct and error DNMS trials were

sorted into their respective categories of: 1) phase cells, which fire differentially during the sample or nonmatch phase; 2) position cells, which fire only during responses on either the left or right lever irrespective of the phase of the task; 3) conjunctive cells, which fire only during a particular combination of position and phase (e.g. left - sample) but not during other possible combinations (e.g. right - sample, right - nonmatch, or left - nonmatch); and 4) trial-type cells, which fire during all task-relevant trials but for only one of the two types of trials (i.e. right sample - left nonmatch or left sample - right nonmatch). All FCTs were characterized based on peak z-scores that were calculated from peri-event histograms ($\pm 1.5s$) around task specific events as follows: 1) The peak bin value (histogram maximum) was found (bin size: 100ms); 2) The mean (M) and standard deviation (S.D) of the background bins (those bins that are equal or less than peak bin value/2) were calculated; 3) peak z-score = (peak bin value – M)/S.D. All FCTs were selected only if the peak z-score around any task specific event was equal or greater than 3.19 ($p < 0.001$). The recruitment of total number of cells, FCTs and individual FCTs across all delay intervals and all trials (including correct and error trials) were assessed using one-way repeated measures ANOVAs for both vehicle and cannabinoid treated groups. Student *t*-tests (paired) were used to assess differences between individual delay intervals.

In addition, peak z-scores of hippocampal principal neurons that showed significant increases in firing during sample and nonmatch responses of correct DNMS trials (all peak z-scores > 3.19) were also analysed and averaged across all sessions. Statistical significance was assessed using one-way repeated measures ANOVAs. Tukey's multiple comparison tests were performed to reveal differences between individual delay intervals.

Results

Increase in cognitive load (delay) causes recruitment of additional hippocampal neurons

The total number of days to achieve performance criterion (80% correct responding per session) and the total number of principal neurons and FCTs activated during task performance were recorded on days when criterion was met. Initial training used 0s delay and was acquired in control rats in approximately 4 days (Fig. 1A). The progressive increase in delay interval to 1–10s, 11–20s and 21–30s was acquired at a similar rate. This was supported statistically with a one-way repeated measures ANOVA failing to show a reliable effect of delay ($F < 1.4$). Progressively expanding the delay, however, caused extra CA3 and CA1 principal cells ($F_{(3,15)} = 18.7$, $p < 0.001$) and FCTs ($F_{(3,15)} = 18.5$, $p < 0.001$) to be recruited. Post-hoc paired *t*-tests confirmed this progressive increase in total number of cells firing at longer delays and the increment in FCTs (see asterisks in Fig. 1B; all *t*'s > 2.7 ; *p*'s < 0.05). Note that the total number of cells refers to all neurons isolated per array during the period of delay acquisition, thus FCTs represent firing correlates that emerge from this pool of cells during higher cognitive demands. Increases in FCTs here refer to the total number of cells recruited across all four categories and not merely to the number of FCT categories *per se*. This is further exemplified in Table 1 which shows that this increase in cell recruitment is a more general phenomenon that occurs across most vehicle-treated animals when subjected to perform the DNMS task at progressively increasing delay intervals. Moreover, it is important mentioning that there were no significant differences in the total number of cells and FCTs isolated between CA3 and CA1 sub-fields (data not shown) suggesting that this delay-dependent recruitment of hippocampal principal neurons occurs equally across both sub-fields of the dorsal hippocampus. Prior studies that have isolated task-specific firing correlates (FCTs) during DNMS performance have found similar results (Hampson et al., 2003; Hampson and Deadwyler, 2000).

To confirm that the recruitment of additional neurons, and in particular FCTs, was a function of increasing cognitive demand, hippocampal CA3 and CA1 neurons were recorded

and FCTs characterized from a separate group of 5 animals to those described in this study, were trained to run the DNMS task without any delay (i.e. 0s delay) for 30 days. Over the 30-day period, the number of recorded neurons increased lightly (Day 1: 9.2 ± 2.3 neurons per animal; Day 30: 10.7 ± 3.1 neurons per animal); however, the neurons identified as FCTs did not differ (Day 1: 5.4 ± 1.8 FCTs per animal; Day 30: 5.9 ± 2.2 FCTs per animal, results not shown). This result suggests that duration of training alone can not account for the increase in FCTs seen in Figure 1

WIN 55,212 exposure impairs delay learning and recruitment of additional hippocampal neurons

In contrast to vehicle-treated animals, WIN-2 treated animals acquire the longer delay intervals only through extended training thereby doubling the number of days to meet criterion at 21–30sec delay compared to 0s. This increase in training days was reliable ($F_{(3,15)} = 18.01, p < 0.001$) and different from 0s at all longer intervals (see asterisks in Fig. 1C; $t's > 2.6$; $p's < 0.05$). Furthermore, a two-way repeated measures ANOVA performed using treatment and delay intervals as factors yielded a main effect of treatment ($F_{(1,24)} = 93.4, p < 0.001$), delay interval ($F_{(3,24)} = 30.3, p < 0.001$) and treatment x delay interval interaction ($F_{(3,24)} = 6.2, p < 0.01$). As expected, post-hoc analysis revealed that the WIN-2 treated animals required significantly more days to achieve performance criterion at 1–10s, 11–20s and 21–30s delay intervals but not at 0s in comparison to controls.

This WIN-2 induced increase in training days was coincident with a failure to recruit additional principal cells and FCTs in CA3 and CA1 sub-fields at long delays. Quite unexpectedly, there was a subtle decline in number of FCTs that were recruited at long delays following the treatment with this cannabinoid (see asterisks in Fig. 1D; $t's > 2.7$; $p's < 0.05$). These results are further represented in Table 2 which shows that the total number of cells recruited is limited across most WIN-2 treated animals compared to those treated with vehicle at progressively increasing delay intervals (*Tab. 1*). Furthermore, *Tab. 2* shows this subtle loss in total number of FCTs recruited across most WIN-2 treated animals with increasing delay intervals (*Tab. 2*). Thus, it is important to note here that these delay-dependent changes in cell recruitment and WIN-2 induced alterations in recruitment occur in almost every animal and is not merely shown by a few selected animals.

WIN-2 suppresses recruitment of all functional cell types

The overall increase in recruitment of FCTs, which was coincident with the increase in delay raises the question, whether all functional cell types experience similar recruitment, or whether some cell types are more task-relevant than others and contribute more significantly to the learning process? A corollary of the observation then is to establish whether WIN-2 selectively affects some FCTs but not others.

We have previously defined four different FCTs (Hampson et al., 1999; Hampson and Deadwyler, 2000) categorized according to specific task-related events. These cell types compose the ensemble code critical for successful DNMS performance. Here we set out to establish the recruitment of the different FCTs (phase, position, conjunctive and trial-type cells) during acquisition learning when delay and thus cognitive load is increased. We observed a delay-dependent increment in number of all 4 types of functional cells in vehicle controls (Figs. 2A & B and 3A & B). Representative examples of different cell types and their peri-event rastergrams and histograms relative to sample and nonmatch responses are also given in Figs. 2 & 3. The recruitment was significant for all cell types in controls (all $F's > 5.3$; $p's < 0.05$), but not in the WIN-2 treated group (all $F's < 2.6$; $p's > 0.05$). Consequently, there was a higher number of the individual FCTs activated at long delays in controls relative to WIN-2 treated rats and this difference was reliable (see asterisks in Figs.

2A & B and 3A & B; all t 's > 2.39; p 's < 0.05). A comparison between controls and WIN-2 treated animals confirmed significant differences in recruitment of additional cells for delays longer than 10 seconds (see asterisks in Figs. 2 & 3). However, performance in WIN-2 animals is readily impaired when delays of 1–10 seconds are implemented (Figs. 1A & C). This appeared to be due to a lack of recruitment of phase cells (Fig. 2A) during this interval since all other FCTs remained stable. For longer delays, and 21–30s in particular, all FCTs are significantly increased in controls; this did not occur in the WIN group.

Chronic cannabinoid treatment suppresses firing during the encoding but not the nonmatch phase of the DNMS task

Finally, we enquired whether cannabinoid treatment would affect the firing of cells during encoding (sample phase) as has been established for fully trained subjects (Hampson et al., 2003; Hampson and Deadwyler, 2000). Figure 4 summarizes the results and confirms that WIN-2 reduced the firing during encoding across all delay intervals (Fig. 4B & C). One-way repeated measures ANOVA performed on hippocampal principal cell activity (peak z -scores) around the sample response (SR) revealed no main effect of delay interval ($F < 1$) in the vehicle group (Fig. 4A & C). By contrast, a significant main effect of delay was observed following WIN-2 treatment ($F_{(3,18)} = 16.02$, $p < 0.001$, Fig. 4B & D) at all delays (q 's > 4.58, p 's < 0.05) relatively to 0s interval. In contrast, one-way repeated measures ANOVAs performed on neuronal activity around the nonmatch response (NR) confirmed that there was no main effect of delay interval in response to WIN-2 treatment ($F < 2.7$, Fig. 5A & B). This effect is further exemplified by peri-event histograms and rastergrams which represent the activity of two CA1 principal cells around the nonmatch response that were recorded across all delay intervals following vehicle or WIN-2 (Fig. 4C & 4D) treatment. The fact that WIN-2 was able to suppress principal cell firing during the sample but not the nonmatch phase across all delay intervals suggests that its chronic effects on encoding phase firing were sustained throughout the entire training procedure showing no signs of tolerance.

Discussion

Having established that hippocampal FCTs represent the encoding of DNMS task-specific information (Hampson et al. 1999a), and that such encoding is essential for correct DNMS performance (Hampson et al. 1997; Hampson and Deadwyler 2000), we examined how such FCTs arise in animals initially learning the DNMS task. While it is possible that the increase in recorded neurons and FCTs illustrated in Fig. 1B could result merely from repeated exposure to the task, this is countered by the fact that animals maintained at 0 s delay for up to 30 days did not show a significant increase in either FCTs or the overall number of cells recorded. The recruitment of additional FCTs when longer DNMS trial delays are experienced suggests that increased cognitive load leads to recruitment of distributed networks of neurons within hippocampus. Thus as DNMS training progresses through different acquisition states, the hippocampus recruits neurons with unique firing correlates (FCTs) to fully represent all of the information necessary to perform the task. This is consistent with our previous recordings (Hampson et al., 1999a) that show repetition of FCTs in distinct segments along the septo-temporal axis of the hippocampus. Moreover, we have demonstrated that lesions of hippocampus produce a delay-dependent deficit in DNMS performance (Hampson et al., 1999b), consistent with findings that near-complete hippocampal lesions are required to impair remote memory, while partial lesions up to 20% still enable normal retention (Martin et al., 2004; Kaut et al., 2003; Moser et al., 1993).

It is important to note that the suppression of FCT recruitment accompanying exposure to WIN-2 is not merely a function of the cannabinoid-induced suppression of sample firing in those FCTs. Acute and chronic exposure to WIN-2 in fully trained animals also suppresses sample firing (Hampson et al. 2000; Hampson and Deadwyler 2003) and results in an

apparent loss of FCTs. However, in the case of fully trained animals, while WIN-2 results in the “loss” of Sample Phase, Left Sample Conjunctive and Right Sample Conjunctive FCTs, it also results in the alteration of Position and Trial-type FCTs to firing patterns equivalent to Left Nonmatch and Right Nonmatch conjunctive FCTs (Hampson and Deadwyler 2003). Thus in fully trained animals the numbers of Position and Trial-Type FCTS are nearly eliminated, Phase FCTs are reduced, and Conjunctive FCTs are actually increased. This is not consistent with Figures 1 & 2 which show that WIN-2 produces no net change in Phase, Position, and Conjunctive FCTs and only decreases the number of Trial-Type FCTs during delay learning.

The results shown here support and extend our previous findings with respect to cannabinoid effects on short-term memory deficits in rats (Hampson et al., 2003; Hampson and Deadwyler, 2000; Heyser et al., 1993) and are consistent with other data probing short-term memory (Suenaga and Ichitani, 2008; Hill et al., 2004; Molina-Holgado, 1995; Fadda et al., 2004). In our DNMS task, hippocampal ensemble firing is represented by at least four task-specific cell categories, which are readily distinguished in fully trained subjects (Hampson et al. 1999b). By contrast, the novelty here rests in the fact that FCTs emerge in a manner dependent on acquisition outcome, i.e. enhancement in cognitive load introduced as progressively longer delays appears to recruit additional task-specific cells exemplified by a positive relationship between delay and number of cells recruited. Administration of WIN-2 restricted the number of task-specific neurons, producing essentially no change relative to the pre-existing number of FCTs, which strongly suggests that exogenous cannabinoids prevent neuronal plasticity in the hippocampus (Hoffman et al., 2007; Misner and Sullivan, 1999) and indeed can hinder the recruitment of new synapses in rat hippocampus (Kim and Thayer, 2001). Consequently, preventing the recruitment of FCTs during learning is a likely mechanism for the impairment in delay acquisition following WIN-2.

Figures 4 & 5 in the current study also confirm that cannabinoids selectively alter hippocampal principle cell firing during the sample phase. It is this selective effect on encoding that likely underlies DNMS behavioral deficits, since (i) sample phase firing is essential for correct behavioral performance (Hampson et al. 2003; Deadwyler and Hampson 2006); and (ii) exogenously applied cannabinoids selectively suppress sample firing in fully trained animals (Hampson and Deadwyler 2003). However, suppression of sample firing alone would not account for the nearly 50% decrease in recruitment of FCTs by the end of training shown in Table 2. Only 30% of FCTs are comprised of exclusively sample phase firing (Figs. 2 & 3). Suppression of sample firing in FCTs comprised of sample plus nonmatch phase firing would leave the nonmatch phase unaffected, resulting in cells that are consistent with Conjunctive FCTs (Fig. 3). Thus the previously reported effects of cannabinoids to suppress sample phase firing could not account for the WIN-2 induced decrease in FCTs shown in Table 2.

The recruitment of FCTs during delay acquisition and their sensitivity to cannabinoids appears to be a specific feature of the hippocampus. One of the intriguing questions regarding hippocampal function is how information is actually processed within the larger context of hippocampus and associated brain areas to encode, retain, and retrieve memories (Hampson et al., 1999; Shapiro and Eichenbaum, 1999; Wirth et al., 2003; Ono and Nishijo, 1999). Recent work done in this laboratory has reported that the hippocampus is involved in encoding selected features of a given stimulus as shown by specific hippocampal neurons that categorize types of visual stimuli presented in a delayed-match-to-sample memory task in monkeys (Hampson et al., 2004).

Previous studies that have attempted to define the differential processing roles of the hippocampus, subiculum and parahippocampal regions, have reported differences in spatial

and temporal encoding (Deadwyler and Hampson, 2004; Anderson and O'Mara, 2003, Golob et al. 2001, Muir and Bilkey, 2003) or object recognition (Fernandez et al. 2002, Moses et al. 2002). While few reports in rodents have studied temporal sequences and other forms of declarative memory in this context (Agster et al. 2002, Eichenbaum, 2000, Manns et al. 2006), studies with non-human primates utilizing more sophisticated cognitive tasks have partitioned memory functions across more widely separated brain regions such as temporal and prefrontal cortices (Freedman et al. 2001, Pesaran et al. 2002, Suzuki and Eichenbaum, 2000, Sakurai and Takahashi 2006). Prefrontal cortical units have been shown to alter their firing during the delay period (Pratt and Mizumori., 2001; Quintana et al., 1988; Kojima et al., 1982) thereby providing a physiological substrate for cross-temporal integration. Although we cannot exclude the possibility that delay acquisition in the DNMS task also engages delay-related firing of dorso-lateral prefrontal units and other connected cortical regions, the hippocampal dependence of the task (Hampson et al., 1999b) suggests that a hippocampal mechanism is a crucial component of acquiring the task. In hippocampus, recruitment of new FCTs appears to be a novel mechanism, by which cross-temporal integration may be established. Despite the blockade in recruitment of encoding-related firing of FCTs at longer delays, animals could still acquire the task when treated with WIN-2. This is not an issue of tolerance, which requires >30 days to occur following WIN-2 treatment (Hampson et al., 2003), but may well be due to an activation of residual cannabinoid-insensitive memory systems. This lends further support to the notion that recruitment of additional hippocampal cells are responsible for the rapid acquisition learning of long-delay DNMS in rats.

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Figure 1.

Repeated cannabinoid administration prior to learning not only slowed the rate of delay acquisition but also hindered the recruitment of FCTs at each acquisition stage. Mean $\pm$ SEM of days to achieve performance criterion and total number of CA3/CA1 principal neurons/FCTs recruited at each delay interval following daily injections of vehicle (A and B) and 0.35 mg/kg of WIN-2 (C and D). (* $p < 0.05$; ** $p < 0.01$).

Figure 2.

Mean ($\pm$ SEM) number of phase (A) and position (B) cells during the session at which animals achieved performance criterion across all delay intervals following daily injections of vehicle or 0.35 mg/kg of WIN-2 respectively. Rastergrams and peri-event histograms of a phase (C) and a position cell (D) recorded simultaneously during the DNMS task. The rastergrams illustrate firing on single trials with a dot each time the cell fired an action potential in relation to left/right sample or nonmatch response. Successive rows depict 50 different trials. The peri-event histograms below the rastergrams reflect the mean firing frequency across 100ms bins.

Figure 3.

Mean ($\pm$ SEM) number of conjunctive (A) and trial-type (B) cells during sessions when animals achieved performance criterion across all delay intervals following daily injections of vehicle and 0.35 mg/kg of WIN-2. Rastergrams and peri-event histograms of a conjunctive cell (C) and a trial-type cell (D) recorded simultaneously during the DNMS task. For details on rastergrams and peri-event histograms, see figure 2.

Sample Response (SR)

Figure 4.

Mean ($\pm$ SEM) of peak z-scores calculated from peri-event histograms of CA3/CA1 principal neurons that demonstrated significant firing around the sample response (SR) following vehicle (A) and WIN-2 (B) treatment across all delays. (C) and (D) represent rastergrams and peri-event histograms of two principal cells that were recorded across the SR phase on days with performance at criterion at each delay following respective treatments. Note the cannabinoid induced suppression in firing during the SR at 1–10s, 11–20s and 21–30s delays, but not at 0s.

Figure 5.

Mean ($\pm$ SEM) of peak z-scores calculated from peri-event histograms of CA3/CA1 principal neurons that demonstrated significant firing around the nonmatch response (NR) following vehicle (A) and WIN-2 (B) treatment across all delays. (C) and (D) represent rastergrams and peri-event histograms of two principal cells that were recorded across the NR on days with performance at criterion at each delay following respective treatments. The cannabinoid failed to suppress firing during the NR.

Table 1

Animal	Vehicle									
	Total number of cells					Total number of FCTs				
	0s	1–10s	11–20s	21–30s	0s	1–10s	11–20s	21–30s		
1	10	12	15	18	7	8	10	15		
2	7	8	14	15	4	5	8	11		
3	12	15	21	23	12	14	19	19		
4	11	12	16	19	4	5	6	9		
5	9	11	13	19	8	8	10	11		
6	11	11	11	11	7	7	7	10		
Total	60	69	90	105	42	47	60	75		

Table 2

		WIN-2							
		Total number of cells				Total number of FCTs			
Animal	0s	1–10s	11–20s	21–30s	0s	1–10s	11–20s	21–30s	
7	6	6	7	8	5	3	3	2	
8	5	7	7	7	5	4	4	4	
9	12	13	13	11	8	8	7	7	
10	9	9	9	9	6	4	4	4	
11	8	10	11	11	6	6	6	5	
12	11	10	10	9	7	6	5	3	
Total	51	55	57	55	37	31	29	25	