RETENTION OF SPECIFICITY OF MEMORY FOR CONTEXT USING REINSTATEMENT

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by

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ABSTRACT

Any learning episode includes a variety of features that range from background context to specific cues that can predict an event. These stimulus attributes are important and play a role in maintaining retention of memory. This study used the Pavlovian fear conditioning paradigm and incorporates reinstatements of the original training context into the design. Experiment 1 had multiple and one reinstatement groups that were tested in the original or shifted context on day 5 of the experiment. Experiment 2 had multiple and one reinstatements groups that were tested in the original or shifted context on day 14 of the experiment. Experiment 3 had multiple and one reinstatement groups that were tested in the original or shifted context on day 6 or day 14 of the experiment. There were significant differences between groups in this study meaning that the multiple reinstatements create a trend toward improvement of memory retention. The implications of this research can apply to a vast majority of human learning and memory research.
Retention of Specificity of Memory for Context using Reinstatement

Any learning episode includes a variety of features that range from background context to specific cues that can predict an event. A memory is a collection of these features that represent specific attributes of an episode or an event. These attributes are conceptualized as a representation consisting of: the stimulus (or stimuli) associated with the outcome, the outcome itself, the response that was made, and the contextual background cues that happen to be present at the time of the episode. The subject notices cues, external or internal, that are similar to the event represented by that attribute to produce a memory (Spear, 1973). Altering stimulating conditions, proactive interference, and retroactive interference have long been recognized as fundamental conditions of forgetting in both animal and human research. (Bouton, 1993; McGeoch, 1932). Altering stimulating conditions refers to a change in the contextual stimuli between training and retention testing. The context is the setting or background in which the event occurs and the room or environment, odors, lighting, temperatures, sounds, internal states and emotions all make-up part of this context. Defining what aspects of a context are important to for memory retention can be difficult.

Research by McGeoch (1932) was one of the first to report that a change in the context or stimulus conditions between training and a retention test was an important determinant of memory retention resulting in a performance decrement. Furthering
McGeoch’s discovery of the importance of context, Thompson and Tulving proposed the encoding specificity hypothesis. According to this theory, when a task is learned the context is incorporated with the learning processes, thus becoming a feature during encoding. At the time of memory retrieval, the context should be as close as possible to the context during the encoding or retrieval process in order to gain optimal performance (Tulving & Thomson, 1973). In a similar vein, Spear suggests the training context matching the testing context is critical for memory retrieval following a delay (Spear, 1973). Therefore, better retention is observed when the context present during training is similar to the context at the time of retrieval. If the context during retrieval is different from the acquisition context, performance is poor. This decrement in performance due to a change in context is known as the context shift effect.

The context shift effect has been demonstrated using a wide range of paradigms in both human and animal research (Anderson & Riccio, 2005; Gordon, McCracken, Dess-Beech, and Mowrer, 1981; Zhou and Riccio, 1996; MacArdy and Riccio, 1991). Many experiments provide clear evidence of the performance deficit due to mismatched contextual stimuli during training and testing. Zhou and Riccio (1996) demonstrated that manipulating the components of a context can disrupt performance in a Pavlovian differential fear conditioning paradigm. In their experiment, rats were trained in a particular room in a certain passive avoidance box. One day after training, subjects were divided into separate groups that received a retention test with a shift in either the room, the box, both the room and box, or no shift in either the room or the box. Subjects tested
in the original room and box had greater retention, meaning more fear in the pavlovian conditioning, compared to those tested with a shift in the room, box, or both. These results show that a shift in components of a context can be detrimental to performance and memory retention.

Gordon et al. (1981) trained rats on an active avoidance task in one context and tested 24 hours later in either the same or different context. Animals tested in the context that differed from the training context performed significantly worse, meaning poorer retention than those tested in the initial acquisition context. Since the task and conditioned stimulus were the same in both contexts, the performance deficit was attributed to the shift in the context. MacArdy and Riccio (1991) used interoceptive stimuli to demonstrate a context shift effect. In their experiment, rats were trained on passive avoidance task in one of two drug states: sodium pentobarbital or chloropent. One day following the training, subjects were tested while in either the same or a different drug state. The important finding from this study was that those subjects tested in the same drug state as training had much better performance, meaning that they showed better retention, than those tested in the different drug state and this has been shown for morphine tolerance as well (MacArdy & Riccio, 1991; Feinberg & Riccio, 1990; Siegel, Hinson, & Krank, 1981).

As previously mentioned, the context shift effect has also been demonstrated in human studies (Riccio, Rabinowitz, & Axelrod, 1994). In 1979, Smith demonstrated the importance of context matching during acquisition and test with college students. Using a
list learning task, students were tested on recall of a list of words. Those students who were tested on recall of the list in the same room in which they learned recalled more words than those tested in a different context (Smith, 1979). Godden and Baddeley (1975) also provide more evidence in the human literature of the context shift effect. Experienced scuba divers learned lists of words while either sitting on the shore by the edge of the water or while 20 feet underwater. The divers were then asked to recall the list of words either in the original or different learning environment. Divers did recall more words when tested in the original learning environment. Borovsky and Rovee-Collier (1990) used 6 month old infants as their subjects to test the context shift effect. They trained the infants to move a mobile by kicking their leg in a crib with a distinct liner. One day following the training, half the infants were tested in the crib with the original liner and half were tested with a novel crib liner. The results were consistent with the context-dependent memory hypothesis that infants tested with the switched liner had poorer retention and kicked significantly less than those tested with the original liner.

The context shift effect has been shown in many different experiments with both animals and humans and seems to be a robust finding; however, it has also been found that there is a paradoxical result when the effects of changing contextual or stimulating conditions on memory are tested following a retention interval between the training and testing. At a short delay, a context shift reliably disrupts performance as we have seen. This performance decrement is demonstrated by a steep contextual stimulus generalization gradient due to the stimulating conditions failing to produce memory
retrieval. When the stimulating conditions are altered shortly after the learning episode the subject is capable of distinguishing between the characteristics of the original and novel stimuli. Introducing the different stimuli produces a discriminable change that disrupts performance. Shifting contextual or conditioning stimuli following a delay attenuates the effect, as reflected in a flatter stimulus generalization gradient. Therefore, the stimulus generalization gradient flattens over time due to the subject forgetting the stimulus attributes and characteristics of the original stimulating conditions. As time passes, novel stimuli become more functionally similar and interchangeable with the stimuli presented during the original acquisition. The consequence of this is that responding becomes under the control of the greater array of stimuli. The forgetting of stimulus attributes produce flattening of the generalization gradient because the subject treats the novel context or stimuli similar to the original stimulating conditions (Riccio, Ackil, & Burch-Vernon, 1992; Riccio & Ebner, 1984; Riccio, Richardson, & Ebner, 1999). The crux of this, referred to as the contextual cues paradox, is that after a long delay there is less disruption from a context shift effect because the animal fails to discriminate between the contexts that leads to the flattening of the generalization gradient with a delay. The contextual change theory states that forgetting is due to subtle changes between the training and testing contexts; the contextual cues paradox arises from evidence that deliberate and explicit modifications of stimulus conditions become less disruptive as a function of time after acquisition (Bouton, Nelson, & Rosas, 1999a;
Bouton, Nelson, & Rosas, 1999b; Riccio, Richardson, & Ebner, 1999; Rosas & Bouton, 1997).

Perkins and Weyant (1958) originally demonstrated that the generalization gradient tends to flatten with a delay between training and testing. In this study, they deprived rats of food so that they could train them with food reward in a black and white runway. After reaching a specified criterion, half the subjects were given a retention test in the original training runway and half were tested in a different runway. They also divided these subjects further with half being tested 24 hours after original training and half tested 7 days later, each in the original or a different context. Of the subjects that were tested 24 hours later, those in the original runway showed good retention, but exhibited poorer retention which was shown by a slower running speed, when tested in the altered runway. However, the same change in context 7 days later had little effect on running speed and retention; the subjects in the altered runway performed as well as those in the original runway. These results demonstrate that the subjects could distinguish between the contexts at the short, but not at the longer delay. This flattening of the generalization gradient was attributed to the forgetting of the training characteristics, leading Perkins and Weyant to conclude that forgetting the color of the runway occurs more rapidly than does the general tendency to run on elevated runways.

Zhou and Riccio (1996) demonstrated the important of multidimensional features of the context in terms of the context shift and forgetting of stimulus attributes. The results of their study (previously described) show not only that changing various
components of the context can result in a deficit, but also that several aspects of stimulus attributes are forgotten over a delay. McAllister and McAllister (1963) found similar findings in their study. Forgetting of stimulus attributes have also been reported with target stimuli as well as contextual stimuli. Thomas and Lopez (1962) also demonstrated flattening of the generalization gradient using discriminative stimuli in a free-operant task using pigeons. The results of these studies demonstrate that changing stimulating conditions between training and testing produces a decrement in performance, the context shift effect, due to the novel context not stimulating memory retrieval. However, this forgetting is attenuated with a delay between training and retention test. Although the mechanisms underlying the forgetting of stimulus attributes are not well understood, there is some evidence that the forgetting can be reversed by reminder treatments (Rosas & Bouton, 1997; Zhou & Riccio, 1994).

Previous research has shown that a brief re-exposure to the context itself shortly prior to testing improved retention, as indicated by the return of the context shift effect. An important finding was that recovery of context specificity was transient which means that the flatter gradient returned if testing was delayed for several hours (Zhou & Riccio, 1994) This shows that manipulation shortly prior to testing alleviates response deficits on a memory task. Using a different paradigm involving the contextual specificity of latent inhibition another study also showed a comparable outcome: the loss of specificity of latent inhibition over a long retention interval was reversed following a brief re-exposure to the context (Rosas and Bouton, 1997).
Another approach to make the transient nature of the recovery of context specificity more persistent is the reinstatement paradigm. Some studies have investigated whether using cueing manipulations would provide a reminder or reinstatement of a previous stimulus exposure, as is commonly the case with forgetting of responses. Ackil et al. showed that forgetting of a CS preexposure (latent inhibition) in a conditioned taste adversion paradigm could be alleviated by a brief exposure to the CS toward the end of the retention interval. A study by Moye and Thomas (1982) found that 24 hours after training, pigeons tested for generalization along a wavelength dimension showed a flatter gradient than those tested immediately. However, the gradient was reinstated by exposing the birds to one of the several training attributes prior to testing.

There is considerable evidence that reexposure to the training context can reduce performance deficits associated with either spontaneous or induced forgetting. In one case, rats trained in a food rewarded T-maze showed a substantial response impairment after a 25-day retention interval, but this forgetting was alleviated by providing a brief exposure to the training context shortly prior to testing (Deweer, 1986; Zentall, 1970). However, this study did not look at the attributes of the stimulus. Zhou and Riccio conducted an experiment using Pavlovian differential fear conditioning to examine the effect of reminder treatments on memory for contextual stimulus attributes. Zhou and Riccio had the aim of determining whether a brief exposure to the training stimulus context after a 1-week retention interval would influence sensitivity to the context shift effect. Their study did show that subjects undergo substantial forgetting of the details of
the training room or context 1 or 2 weeks after training, and that the two contexts become interchangeable. Perhaps the most important finding of their study was that the forgetting of stimulus attributes is also reversible. Either 1 or 2 weeks after training, when the subjects have forgotten these details of the training context, a re-exposure to the training context led the subjects to show the generalization gradient again. In both experiments, subjects that were given the 90 second reexposure to the training context shortly before testing exhibited significantly shorter test latencies in the shifted room than that of the subjects tested in the original room. It appeared that from this study when subjects have forgotten the details of the training context, reexposing them to some attributes of the training context reactivates the corresponding memory trace.

Another line of research looks at the response memory as opposed to memory for stimulus attributes. In this paradigm, reinstatement is a small amount of partial practice or repetition of an experience over the developmental period, which is enough to maintain an early learned response at a high level, but not enough to produce any effects in animals that have not had the early experience. Campbell and Jaynes trained albino rats using fear conditioning in the passive-avoidance chamber. Twenty-five day old rats were placed on the black side of the chamber during training and received 15 2-second shocks on a 20-second variable interval schedule. The animals were removed and placed on the white side for 5 minutes and then the procedure was repeated. Thus each animal received 30 shocks during training. Reinstatements were then given to some of the animals 7, 14, and 21 days after training. The reinstatements consisted of placing the animal on the black
side of the chamber and giving them a brief 2-second shock at a random number of seconds during a one minute interval, then placing the animal on the white side for one minute. At test the rats were placed on the black side and allowed to roam freely to either side for an hour duration. The total time spent on the white side was recorded. The group that had received the early fearful experience followed by the reinstatement shocks spent an increasing percentage of its time on the white side. The control group that had the early fearful experience, but no reinstatements, did not show significant fear of the black side. The control group that had not had early fearful experience, but had received the three brief shocks over the month, also failed to acquire any significant fear of the black side. This shows that there is some small amount of practice over certain time intervals which could maintain a previously learned response and yet not be enough to train animals to perform that response (Campbell & Jaynes, 1966). Evidence from research on human infants also suggests that periodic re-exposure to an abridged form of the original training context can enhance long term retention of the response memory. In one experiment, 3-month old infants learned to move a crib mobile by kicking and subsequently received one, two, or three reinstatements. Each reinstatement was a partial training episode, but was only one-sixth of the duration of the original training. They found that presenting a single reinstatement when the memory was inactive failed to recover the response memory 1 day later, however; increasing the number of reinstatements to two or three when the memory was active prolonged retention. Although 3-month old infants typically remember for less than 6 days, after 3
reinstatements they were able to exhibit retention 6 weeks after training. Their results demonstrated that periodic reinstatements can maintain young infants’ retention over long delays (Galluccio and Rovee-Collier, 1999; 2005). The theoretical framework for the current study is based on the reinstatement phenomenon.

Experiment 1

An alternative approach to identifying processes that control the forgetting of stimulus attributes is to explore manipulations that may help to maintain the target information. This study, patterned after the classic “reinstatement” paradigm, looks at which decrements in subjects are prevented by periodic exposure to an abridged form of the training trials. Also, one purpose of this thesis study is to investigate a basic memory phenomenon, the forgetting of stimulus attributes instead of the memory for the response. Many studies focus on impairments of retention as we have seen; however, another way to address the processes involved in memory for stimulus attributes is to investigate what factors serve to maintain retention of the discriminative properties of stimuli. Based on previous research in this area, one theoretical issue of practical importance is whether the retention of stimulus memory for a context can be increased. Therefore, this study examines whether occasional re-exposures to the training context “reinstatement” will maintain memory for attributes without any further CS-UCS presentations. Evidence for maintaining attribute memory would extend the concept that repeated retrievals establish multiple retrieval contexts that enhance long term retention. The major goal of the current study is to determine whether occasional re-exposures to the training context will
maintain the memory for those attributes. It is hypothesized that these reinstatements, or periodic reactivations, seem more likely to occur in human and animal memory processes. These reinstatements would keep the memory for stimulus attributes active and it may not cause the stimulus generalization gradient to flatten over time. This may lead to resolution of the contextual cues paradox (previously described). Previous studies showing that reminders just before test reestablish the context shift effect was not a compelling resolution to the paradox because in the "real world" it is unlikely that people routinely have pre-exposures to the training context just before recall. Therefore, the hypothesis for this study is that the multiple reinstatement group and the one reinstatement group should perform better on the task at the retention test regardless of context.

METHOD

Subjects

Subjects were 49 male Long-Evans rats, approximately 90 days old at the start of the experiment. Animals were singly housed in the Kent State University animal colony in a rectangular transparent plastic box (45.7 (L) x 25.4 (W) x 20.3 (H) cm). Animals were on a 15/9 light/dark cycle throughout the experiment and were provided ad lib food and water throughout the experiment. All experiments took place during the light portion and at the same time each day.
**Apparatus**

Two different rooms and passive avoidance chambers were used in the experiment. Context A was a 1.83 x 2.74 m room painted white. Posters were placed on each wall to provide extra visual cues since the Long-Evans rat species are known to have adequate vision perception. The room was illuminated by a standard 40w bulb. Context A also had an olfactory cue provided by a Glade clean linen air freshener for a distinguishable odor. Context B was a 1.83 x 2.74 m room painted white. Posters were also placed on each wall to provide different, extra visual cues. The room was illuminated with a 25w red light. Context B has no odor, white noise for background noise, and normal lighting. Training and testing were conducted in a 43.18 (L) x 17.78 (W) x 17.78 (H) cm black-white Plexiglas shuttle box with a grid floor (2mm grids spaced at 1mm apart center to center). The two compartments were equal in size and divided by a guillotine door. The white compartment consisted of a transparent Plexiglas lid, with Plexiglas walls. The black compartment had a black lid and black walls. Footshocks were delivered through the grid floor via a constant current AC shock generator. (Model 5806 Lafayette Instruments Co., Lafayette, IN).

**Procedure**

All subjects were handled for three days prior to training. This study used Pavlovian differential fear conditioning in the passive avoidance chamber. This type of learning involves the pairing of a neutral (harmless) cue, which in this case is the black
compartment of the apparatus, with foot shock. During conditioning/training, the subject was originally placed on the white side of the black/white chamber. After 10 seconds on the white side the door was opened and the rat was allowed to cross to the black side. This latency to cross was recorded. Once the rat had crossed, the door was closed and it received 2 brief 1 second inescapable shocks of 0.5 mA intensity 5 seconds apart. This conditioning was then followed by a reinstatement reminder of the original conditioning context.

The reinstatement exposures involved placing the rat back into the chamber for either 90 or 270 seconds without shock. The re-exposure was given in the original context A. Testing in the original context A or a new context (B) occurs on day 5. The experimental group received training and multiple reinstatements (three evenly spaced reminders). A forgetting control group that did not receive any reinstatements was trained in context A and then tested in context A or B. This allowed for the assessment of the baseline of fear and to evaluate the effectiveness of the reinstatement exposures. Handling effects were accounted for between the groups. An exposure control received training and then reinstatement reminders on day 2 (all three reminders at once). This set of conditions generated 3 retention intervals for each of the 3 groups, using 2 distinct contexts, A or B. The study design of all conditions are displayed in table 1. Testing involved behavioral observations which include latency scores as well as total time on the safe side during a ten minute period without shock. Maintained memory of stimulus attributes was reflected in short latencies and/or less time spent in the safe area.
Therefore, a successful passive avoidance response was when the rat refrains from entering the black side which means that the memory was reactivated. The number of rats in each group in the study were nine. With rats randomly assigned to the six groups and given the usual degree of variability among rats and based on the past experience with similar studies this was a good number of subjects in a group and allowed adequate power to reject the null hypothesis if there was an experimental effect.

RESULTS

Training latencies were first evaluated in a simple one-way ANOVA to show that all the animals exhibited short step-through latencies and strong preference to the black compartment at training. The ANOVA showed there were no significant differences among the training latencies for the six groups ($F(5,43)=2.081, p<.05$).

A 2x3 factorial ANOVA revealed that for the mean total time white measure there was a significant main effect for context ($F(1,43)=27.173, p<.05$). The mean total time on white side scores for each group can be seen in Figure 1 based on context at test. Post-hoc tukey test revealed that in regards to context the one reinstatement group differed from forgetting control ($p<.08$).

The mean test latency scores yielded a significant main effect for both context ($F(1,43)=27.026, p<.05$) and group ($F(2,43)=5.935, p<.05$). The mean test latency scores for each group are shown in Figure 2 based on context at test. A post-hoc Tukey test for context showed that the difference was between the one reinstatement group and
the forgetting control (p< .05). The mean test latency scores for each group are shown in figure 2 based on context at test.

DISCUSSION

The results show some important aspects of this study. The first aspect is the reinstating of the context shift effect. For all groups tested in context A, better retention was shown in more total time on the white side and longer latency scores than any groups tested in context B reflected in the main effect for context.

There was a main effect for context and a main effect for group when looking at the mean latency scores at test. This means that the latency scores were affected by which group the animal was in as well as which context the animal was tested in. When tested in context A all groups had relatively similar retention. The one reinstatement group was significantly different from the forgetting control, meaning that the one reinstatement group did exhibit shorter latencies but the difference from context A to context B was less than that of the forgetting control.

It is important to remember that good retention of memory for attributes is seen by poor performance in the shifted context because the rats are discriminating Context B from Context A. The data obtained indicates that the multiple and one reinstatement groups did not have a detectable effect on attribute memory retention regardless of context. Therefore, what is of importance is why the reinstatements did not serve as better reminders of the training episode. It may be of interest to look at whether the distribution
of the exposures is important or whether the cumulative amount of exposure is important here. Another possibility for future study is to see how long the retention interval can be extended after the last exposure. Some limitations to this experiment are that it used only male rats, and had only a 5-day study design. This is a somewhat arbitrary interval; it might be that under the present conditions, it takes longer for the contextual gradient to flatten. This suggests that for some reason the reminders or “reinstatements” of the context were not serving to increase retention on the learning task. Further investigation of this effect is necessary.

A post-hoc power analysis revealed that fifteen rats would have been needed in each group in order to have adequate power (0.80) for a large effect size. Power level in the current experiment was 0.495 due to smaller sample sizes, which may have had an effect on the results.

Experiment 2

In order to find a reinstatement effect, in which the controls show relatively similar responding in the two contexts, but the experimental group shows differential performance, another experiment was conducted. Based on experiment one, this second experiment lengthened the retention interval. In addition, I attempted to determine whether the distribution of exposures or the cumulative amount of exposure is important in producing a reinstatement effect. The design of this experiment was similar to that of the previous experiment. The main changes from the previous experiment are that the
duration of the study is longer and the amount of time for the reinstatement exposures are
decreased. These changes were made to show how long the effect lasts and also if the
multiple reinstatements maintain stimulus memory compared to that of both the
forgetting controls and the one reinstatement group.

METHOD

Subjects

Subjects were 54 male long-Evans rats, approximately 90 days old at the start of
the experiment. Animals were singly housed in the Kent State University animal colony
similar to that of the previous experiment. All experiments took place during the light
portion and at the same time each day.

Apparatus

Two different rooms and passive avoidance chambers were used in the
experiment. The contexts and apparatus were the same as previously mentioned in
Experiment 1.

Procedure

The general design was similar to that of the first experiment with a few changes.
Pavlovian differential fear conditioning in the passive avoidance chamber in which the
black compartment is associated with footshock was followed 2, 4, 6, 8, 10, and 12 days
later by a reinstatement reminder of the original conditioning context. This re-exposure
was given in the white side of the chamber for 60 sec. in the original context A. Testing
in the original context A or a new context (B) occurred on day 14. This experimental group received training and multiple reinstatements (six evenly spaced reminders). A forgetting control not receiving any reinstatements was trained in context A and then tested in either context A or B. This allowed us to assess the baseline of fear and evaluate the effectiveness of the reinstatement exposures. Another control will receive training and then one reinstatement reminder just on day 12 to see if the earlier exposures were necessary for memory retention (equal amount of time for reminder as other group but all on day 12). This set of conditions generated 3 retention intervals for each of the 3 groups, using 2 distinct contexts. The study design and all conditions are depicted in table 2.

RESULTS

Training latencies were first evaluated in a simple one-way ANOVA to show that all the animals exhibited short step-through latencies and strong preference to the black compartment at training. The ANOVA showed there were no significant differences among the training latencies for the six groups (F(5,48)=0.180, p=.05).

A 2x3 factorial ANOVA revealed that for the mean total time white measure there was a main effect for context approaching significance (F(1,48)=3.207, p< .08). The mean total time on white side scores for each group can be seen in Figure 3 based on context at test.
The mean test latency scores non-significant results for both context and group. The mean test latency scores for each group are shown in figure 4 based on context at test.

DISCUSSION

The results from this study show null effects; however, it is important to note that even though the results were not significant there were some changes in the anticipated direction. There is a trend for the multiple reinstatement group and the one reinstatement group to show somewhat better retention than the controls. Even though the differences between the reinstatements groups and the controls were not significant, the numerical differences encouraged further study on this topic. Once again, adjusting the amount of time of the exposures and duration of intervals is important to finding the parameters that are going to show us what will help to maintain the memory.

A post-hoc power analysis revealed that fifteen rats would have been needed in each group in order to have adequate power (0.80) for a large effect size. Power level in the current experiment was 0.547 due to smaller sample sizes, which may have had an effect on the results.

Experiment 3

Based on the results of the previous two experiments experiment 3 will further investigate the parameters of the memory for stimulus attributes. The study design for the experiment differs from the previous experiments because the length of the retention
interval will be included as a manipulation. Some groups will be tested at 6 days and some groups will be tested at 14 days. The reinstatement exposures will be of the same duration as the previous experiment, but the number of exposures will be manipulated. Some groups are tested in context A and some will be tested in context B as before.

METHODS

Subjects

Subjects were 36 male long-Evans rats in the experimental conditions and 24 make long-Evans rats in the control conditions, approximately 90 days old at the start of the experiment. Animals were singly housed in the Kent State University animal colony similar to that of the previous experiments.

Apparatus

Two different rooms and passive avoidance chambers were used in the experiment. The contexts and apparatus were the same as those mentioned in the previous experiments. The control conditions were handled instead of reinstated to account for handling effects, then they were tested at either 6 or 14 days in either context A or B.

Procedure

The general design is similar to that of the first experiments with some changes. Pavlovian differential fear conditioning in the passive avoidance chamber in which the black compartment is associated with footshock was used. Experiment 3 consisted of
manipulating the number of reinstatements and the duration of the study. There were 10 groups used in this study. The study design and all conditions are displayed in table 3. These groups were trained in Context A. Groups were tested in context A or B on either Day 6 or Day 14 of the experiment. Control groups were trained, but received handling instead of reinstatements. They were then tested 6 or 14 days after training in either context A or context B as mentioned above. It is important to note that these subjects were run at a different time than the experimental groups because of subject availability. There is no reason to believe that the conditions were different from those for the experimental groups.

RESULTS

Training latencies were first evaluated in a simple one-way ANOVA to show that all the animals exhibited short step-through latencies and strong preference to the black compartment at training. There was no significant difference among the training latencies for the six experimental groups (F(5,30)=1.323, p=.05), nor were there any significant differences in training latencies for the four control groups (F(3,20)=0.378, p=.05).

A 2x2 factorial ANOVA for the 6-day test interval revealed that for the mean total time white measure there was a significant main effect for context (F(1,20)=4.335, p<.05) and a significant main effect for group (F(1,20)=8.659, p<.05). The mean total time
on white side scores for each group can be seen in Figure 5 based on context at test. There was also an interaction of context and group (F(1,20)=5.481, p< .05).

The mean test latency scores revealed significant results and a main effect for both context (F(1,20)=3.331, p< .08) and group (F(1,20)=5.707, p< .05). The mean test latency scores for each group are shown in figure 6 based on context at test.

A 2x3 factorial ANOVA for the 14-day interval revealed that for the mean total time white measure there were non-significant results. The mean total time on white side scores for each group can be seen in Figure 7 based on context at test.

The mean test latency scores revealed a main effect for group approaching significance (F(2,30)=2.898, p< .07). The mean test latency scores for each group are shown in figure 8 based on context at test. Post-hoc tests further showed a trend that the one reinstatement group differed from the forgetting control approaching significance (p< .09).

DISCUSSION

The 6-day reinstatement group that was tested in context B showed less retention than that of the controls. This is what was expected due to the fact that longer latencies in the shifted context would reflect forgetting of stimulus attributes. However, since the latencies for the shifted one reinstatement group are shorter than that of controls we see that subjects are discriminating between the contexts. This is also true for the 14-day interval however, as seen from the results this can not be attributed to just the reinstatements, because the controls retention increased as well. While the reinstatement
groups did have improved retention, why the control groups reached a ceiling effect is of interest for further analysis. The ceiling effect shows us that the control groups were treating the contexts as if they were the same instead of discriminating between the two and still had a large amount of retention of the fear. Non-parametric tests as well as median scores were looked at because of the smaller sample sizes and the appearance of what looked like a ceiling effect; however, these data are not included because they were not significant.

A post-hoc power analysis revealed that eleven rats would have been needed in each group in order to have adequate power (0.80) for a large effect size. Power level in the current experiment was 0.465 due to smaller sample sizes, which may have had an effect on the results.

GENERAL DISCUSSION

This set of experiments attempted to examine whether occasional re-exposures to the training context or “reinstatements” would maintain the memory for the stimulus attributes without using any other CS-US presentations. While many researchers have investigated the effects of training context reminders in many condition-response paradigms showing that reminders just before test re-establish the context shift effect. In the real world it is unlikely that people routinely have pre-exposures to the training context just before recall. However, something akin to periodic reactivation seems more
likely. Therefore, this study was furthering their investigations by focusing on some of
the characteristics of reinstatements before test and their effect on memory.

In experiment 1, the hypothesis was that the multiple reinstatement group
and the one reinstatement group should perform better on the task at the retention test
regardless of context. The results show that the reinstatement groups (both multiple and
one reinstatement) did not show better retention than the controls, even when the data
were combined for these groups. This suggests that for some reason the reminders or
“reinstatements” of the context were not serving to increase retention on the learning
task. A trend was found that the multiple reinstatement group, even though it did not
show more retention than the controls, did show more retention than the one
reinstatement group in context A or context B. Also, all testing in context A for the
multiple reinstatement, one reinstatement, and forgetting controls showed better retention
than any of the groups tested in context B. This result is consist with previous studies in
the literature that have obtained the context shift effect using reminders (Gordon et al,
1981; Zhou & Riccio, 1994; MacArdy & Riccio, 1991). However, the data obtained lead
us to obtain the null hypothesis that the multiple and one reinstatement groups did not
have an effect on retention regardless of context. Therefore, one question is why the
reinstatements did not serve as better reminders of the training. The multiple
reinstatements did help retention compared to that of the one reinstatement group, as
mentioned above, so this led to the question of whether the distribution of the exposures
is important or whether the cumulative amount of exposure was important.
A major limitation of this study was the 5 day study design. This was an arbitrary interval and it is hard to know in advance how quickly the contextual attributes will be forgotten and it might be that it takes longer for the gradient to flatten. Perkins and Weyant (1958) used 24 hours after test and then looked at 7 days later to see the effects of the context shift. Galluccio & Rovee-Collier (1999) looked at one-day and then 6 weeks later. It has been shown that the longer delay between the original training and the testing produces a flattening of the generalization gradient due to the forgetting of stimulus attributes; therefore, a longer delay may show the flatter gradient that we need to evaluate reinstatement. Since there is not much forgetting of stimulus attributes it is hard to see the recovery of memory (Bahrick, Clark, & Bahrick, 1967; Borovsky & Rovee-Collier, 1990; MacArdy & Riccio, 1991; McAllister & McAllister, 1963; Perkins & Weyant, 1958; Thomas & Lopez, 1962; Zhou & Riccio, 1996).

Therefore, experiment 2 was conducted to further explore which conditions are necessary to enhance the long term retention using reinstatements. The main changes from experiment one were that the duration of the study was now extended to 14 days. The amount of time of the reexposures was decreased since the duration of the study was increased. The results of experiment 2 revealed null effects; however, it is important to note that even though the results were not significant there are some improvements in this experiment compared to the previous experiment. There is a trend for the multiple reinstatement group and the one reinstatement groups to show somewhat better retention than the controls. Even though the differences between the reinstatements groups and the
controls are not significant, it suggested that other parameter values might be more effective.

The study design for experiment 3 differed from the previous experiments because the length of the retention interval was used as a manipulation. Some groups were tested at 6 days and other groups were tested at 14 days. Experiment 3 yielded some significant results that are important because of what they suggest. It was assumed from previous literature and work done in the Riccio laboratory that both the multiple reinstatements and one reinstatement would have an effect on maintaining memory. The results found in this experiment resemble somewhat the findings reported in the literature. The 6-day forgetting controls tested in B had significantly longer latencies than the one reinstatement group also tested in B. This shows that the one reinstatement group had maintained the memory for the stimulus attributes since there was not a loss of discrimination between the contexts. With one reinstatement and multiple reinstatements at 14-day interval there are significant differences in retention. The one reinstatement group tested in context B showed better retention than that of the control. The multiple reinstatement group tested in context B also showed better retention than that of the control. The context shift effect was found in the control groups that did not receive any reinstatements. The forgetting control tested in context B showed impaired retention compared to that of the forgetting control tested in context A. Re-exposure to the training context did lead subjects to show generalization; however, in comparison to Zhou and Riccio (1996) there was not an overall strong effect for the reminder treatments over the
control groups. There were trends that showed the reinstatements groups had better retention than the controls, but this effect was not seen in all the experiments. One reason could be that the parameters of the experiment were not appropriate. This study allowed for the examination of what features of the context are important. The parameters are the necessary and sufficient conditions in which the reinstatements will act to maintain retention of the memory. Each experiment had its own limitations and overall this study had several limitations that may have contributed to these results. The number of rats for the third study made the sample sizes small in comparison to other studies.

Groups that did not receive reinstatements were handled to account handling effects. However, handling effects may also play a role in reducing the effectiveness of the reinstatements. They may serve as a cue for extinction or may even provide a cue for more learning. There is also research that indicates sex differences in handling effects (Craft, Clark, Hart, & Pinckney, 2006). Handling effects may play role in the results obtained from these studies.

The reminder treatments should serve to alleviate the deficit found in the subjects’ performance when the contexts are shifted. Another implication noted by Campbell and Jaynes (1966) was that reinstatement could be a major mechanism by which effects of early experiences are incorporated to memories for later in life. They go on to say that there are three developments that can occur after a pleasant or unpleasant event. The first is that the experience may gradually be forgotten. The second is that it may be remembered and persist if reinstated. The third is that it undergoes maintenance where it
is either forgotten or parts are repressed. The subjects in these experiments seem to be involved in third development. It is evident that the controls groups are still remembering the original training and there may have not been enough time for them to forget yet. However, Campbell & Jaynes experiment examined the reinstatement of the fear response, which is a great distinction from the reinstatement of memory for specific context stimulus attributes, which were of interest in the current study. (Campbell & Jaynes, 1966). However, the idea of reinstatement is important and there are several other research questions that can be looked at using this reinstatement paradigm to increase of knowledge of what effects the reinstatements have: How long can the retention interval can be extended after the last exposure? Would an abbreviated form of conditioning at the time of re-exposure be more effective for the maintenance of the attribute memory? Are there gender differences? What effect would interference from multiple contexts have on memory retention? Would different drug states effect attribute memory? These and other questions can be explored once the parameters allow for the effect of reinstatements on maintaining the memory for events with stimulus attributes to be found and replicated.

The context shift effect has been shown in many different experiments with both animals and humans and seems to be a robust finding; however, it has also been found that there is a paradoxical result when the effects of changing contextual conditions on memory are tested following a retention interval between the training and testing. At a short delay, a context shift reliably disrupts performance as we have seen. This
performance decrement is demonstrated by a steep contextual stimulus generalization gradient due to the new stimulus failing to produce memory retrieval. When the stimulating conditions are altered shortly after the learning episode the subject is capable of distinguishing between the characteristics of the original and new stimuli. Shifting contextual stimuli following a longer delay attenuates the effect, as reflected in a flatter stimulus generalization gradient. Perkins and Weyant (1958) originally demonstrated that the generalization gradient tends to flatten with a delay between training and testing. Therefore, the stimulus generalization gradient flattens over time due to the subject forgetting the stimulus attributes and characteristics of the original conditions. So, the crux of this paradox is that after a long delay there is less disruption from a context shift effect. A resolution to this paradox seems possible by looking at the stimulus attributes effect on memory retention. Stimulus attributes provided during the training and reinstatements may include those of the training context, not just those of the unconditioned stimulus because reactivation exposures produce memory recovery without direct retraining. Impaired retention of stimulus attributes would provide a basis for the weakening of responding typically measured as retention loss. More specifically, during the retention interval, generalized stimuli in the environment become increasingly capable of eliciting response tendencies or a representation of the training event. Reinstatements could reduce the probability of responding on the retention test.

Continued research in this area will show us the effect that periodic reactivation treatments would have on extending the ability of an internal or external context shift to
disrupt performance at a long test delay. Reactivations that occur periodically and keep the memory active could help resolve the cues paradox. These reinstatements under the right parameters should be able to enhance the memory for stimulus attributes at the short retention interval as well as a longer retention interval. This can then lead to a resolution of the cues paradox (Bouton, Nelson, & Rosas, 1999a; Bouton, Nelson, & Rosas, 199b; Millin & Riccio, 2004; Riccio, Richardson, & Ebner, 1984; Rosas Bouton, 1997).
<table>
<thead>
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<th>Group</th>
<th>Exp.</th>
<th>Reinstate</th>
<th>Train-</th>
<th>Context</th>
<th>Exposure</th>
<th>Test-</th>
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Table 1. Study design for experiment 1. The six groups in the experiment are listed along the left side and the days of the experiment are listed at the top. This table shows what occurred in regards to experimental procedure each day.
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<td>Test-Context A</td>
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Table 2. Study design for experiment 2. The six groups in the experiment are listed along the left side and the days of the experiment are listed at the top. This table shows what occurred in regards to experimental procedure each day.
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<th>Day 10</th>
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<td>Handle for 30 sec.</td>
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<td><strong>Group 3</strong> one reinstatement 6 day A-A</td>
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<td><strong>Group 5</strong> Multiple reinstatement 14 day A-A</td>
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<td><strong>Group 6</strong> Multiple reinstatement 14 day A-B</td>
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<td>Reinstate 60 sec.</td>
<td>Test-Context B</td>
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Table 3. Study design for experiment 3. The ten groups in the experiment are listed along the left side and the days of the experiment are listed at the top. This table shows what occurred in regards to experimental procedure each day.
Figure 1. Mean TTW (±SEM) on Experiment 1 for one reinstatement, forgetting control, and multiple reinstatement groups based on context at retention test.
Figure 2. Mean Test Latency Scores (±SEM) on Experiment 1 for one reinstatement, forgetting control, and multiple reinstatement groups based on context at retention test.
Figure 3. Mean TTW (±SEM) on Experiment 2 for one reinstatement, forgetting control, and multiple reinstatement groups based on context at retention test.
Figure 4. Mean Test Latency Scores (±SEM) on Experiment 2 for one reinstatement, forgetting control, and multiple reinstatement groups based on context at retention test.
Figure 5. Mean TTW (±SEM) on Experiment 3A for reinstatement and forgetting control groups based on context at retention test at 6-day interval.
Figure 6. Mean Test Latency Scores (±SEM) on Experiment 3A for reinstatement and forgetting control groups based on context at retention test at 6-day interval.
Figure 7. Mean TTW (±SEM) on Experiment 3B for one reinstatement, forgetting control, and multiple reinstatement groups based on context at retention test at 14-day interval.
Figure 8. Mean Test Latency Scores (±SEM) on Experiment 3B for one reinstatement, forgetting control, and multiple reinstatement groups based on context at retention test at 14-day interval.
REFERENCES


