The Effects of FG7142 on Two Types of Forgetting in 18-Day-Old Rats

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The authors studied the role of gamma-aminobutyric acid (GABA) in 2 types of forgetting of fear in the developing rat. One type of forgetting studied was that observed after an intermediate retention interval (the “Kamin effect”); the other type studied was that observed after a longer interval (infantile amnesia). Rats were given pairings of an auditory conditioned stimulus with shock, and learned fear was assessed by freezing. Forgetting at an intermediate retention interval (1 hr) was not alleviated by the GABA$_{A}$ receptor partial inverse agonist FG7142 (0, 1, 5, or 10 mg/kg), whereas forgetting at a longer retention interval (48 hr) was alleviated. These results suggest that in the developing rat, forgetting observed at different retention intervals is mediated by different physiological mechanisms.

**Keywords:** Kamin effect, infantile amnesia, FG7142, forgetting, GABA

Forgetting and memory are intricately related processes. Understanding one of these processes should aid in the understanding of the other. Further, a complete understanding of either process will require an understanding of the other. Considerable progress has been made in delineating the neural bases of learning and memory over the last 2 decades. There is now compelling evidence that common neurobiological mechanisms underlie seemingly disparate memory systems in a wide range of species and tasks (e.g., the withdrawal reflex in *Aplysia*, olfactory conditioning in *Drosophila*, and spatial and contextual conditioning in rodents; for reviews, see Abel & Kandel, 1998; Kandel, 2001). In stark contrast, considerably less attention has been paid to the neural bases of forgetting.

Forgetting is observable at two critical time points creating a nonmonotonic memory retention function. Specifically, rats tested immediately (1–10 min) after training or at some later time (24 hr) typically exhibit good retention, whereas rats tested at intermediate (1–6 hr) intervals do not (Kamin, 1957). This poor retention at intermediate intervals is often referred to as the Kamin effect. In a recent demonstration of this phenomenon, McNally and Westbrook (2003) assessed contextual fear in adult rats, as measured by freezing, and found that the level of freezing was significantly lower when the test occurred after a 6-hr retention interval compared to either a 2-min or 24-hr interval. Likewise, Pugh and Rudy (1996) conditioned contextual fear to a highly salient context in 23-day-old rats and documented significantly lower levels of conditioned freezing when rats were tested 10 min after conditioning compared to testing either immediately or 24 hr after conditioning.

The Kamin effect is a robust phenomenon that has been observed across a wide range of species (e.g., goldfish, octopus, honeybee) with variations in the temporal position at which maximal forgetting occurs (see Sutton, Masters, Bagnall, & Carew, 2001). It therefore represents a fundamental forgetting phenomenon (Sutton et al., 2001).

The second critical time point for forgetting is after a prolonged interval. Although adult rats can exhibit retention over very long intervals (Gale et al., 2004), infant rats typically forget quite quickly; this more rapid rate of forgetting observed in infant rats is referred to as infantile amnesia (Campbell & Spear, 1972). For example, Kim, McNally, and Richardson (2006) subjected 18-day-old rats to three pairings of an auditory conditioned stimulus (CS) and a shock unconditioned stimulus (US). When tested 1 day later, these rats exhibited good retention, as reflected in high levels of CS-elicted freezing. By contrast, when they were tested 10 days later, substantial forgetting was observed (i.e., the CS elicited much less freezing). In a subsequent study, 16-day-old rats given two CS–US pairings were found to exhibit substantial forgetting when tested only 2 days after training (Kim & Richardson, 2007). At least one neural substrate underlying infantile amnesia is gamma-aminobutyric acid (GABA). GABA is the major inhibitory neurotransmitter in the mammalian central nervous system (Wong, Bottiglieri, & Sneed, 2003); GABA$_{A}$ receptors are particularly abundant in the amygdala, the central structure of the fear system (Davis, Campeau, Kim, & Falls, 1995). Forgetting of a successfully encoded memory can be caused by a storage failure or a retrieval failure. For forgetting to be alleviated, the memory must be retained (i.e., retrieval failure), and forgetting must be due to a form of inhibition. Consistent with this notion, both Kim et al. and Kim and Richardson reduced the inhibition due to GABA and successfully attenuated infantile amnesia by a pretest injection of the GABA$_{A}$ partial inverse agonist FG7142. Taken together, these results show that this forgetting is due to the imposition of a GABAergic mask that impairs retrieval of the fear memory.

The Kamin effect and infantile amnesia represent examples of forgetting due to a retention interval. The Kamin effect occurs in the hours immediately following training and has been viewed as...
a decline in short- or intermediate-term memory (Kamin, 1957; Rudy & Wright-Hardesty, 2005; Sutton & Carew, 2002). In contrast, infantile amnesia occurs in the days to weeks following training and is considered a decline in long-term memory. The similarities or differences in their mechanisms remain to be elucidated. In addition, it is unclear whether these two types of forgetting reflect the decline of two distinct memory traces or the fluctuating course of a single memory trace. Given the evidence that infantile amnesia is due, at least in part, to the imposition of a GABAergic mask, the evidence that the forgetting at the two time points is a consequence of a single memory trace that fluctuates in strength. However, if the Kamin effect is not alleviated by the reduction of GABA activity, then the decline in short-term memory may be due to inhibition by another mechanism, highlighting the differences between the traces of short- and long-term memory; or it may be due to the decay of short-term memory. Consistent with previous evidence, alleviation of infantile amnesia was expected following the pretest administration of the GABA\textsubscript{A} partial inverse agonist FG7142.

General Method

Subjects

Subjects were experimentally naive male and female Sprague-Dawley rats bred and housed in the School of Psychology at the University of New South Wales. Rats were housed in plastic boxes (24.5 cm long $\times$ 37 cm wide $\times$ 27 cm high) covered with wire lids, with their mother, in litters of eight. No more than 1 rat per litter was allocated to any given group. Rats were 18 ($\pm$1) days of age when trained, except for rats in Experiment 2B, which were 16 ($\pm$1) days of age at training. All rats were the same age (i.e., 18 days) at the time of test. Rats were maintained on a 12-hr light–dark cycle (lights on at 0600) with food and water freely available. All rats were treated according to the principles of animal use outlined in The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th ed.; National Health and Medical Research Council, 2004), and all procedures were approved by the Animal Care and Ethics Committee at the University of New South Wales.

Drugs

FG7142 (\(\beta\)-Carboline-3-carboxylic acid N-methylamide; Sigma-Aldrich, Sydney, Australia) was mixed in 0.9% wt/vol sterile saline with one drop of Tween 80 added per 5 ml of saline. An equivalent amount of Tween 80 was added to the sterile saline solution injected as a control. All injections were subcutaneous (nape of the neck), at a volume of 2 ml/kg, and occurred 10 min prior to test.

Apparatus and Procedure

Conditioning occurred in a rectangular (30 cm long $\times$ 30 cm wide $\times$ 23 cm high) Plexiglas chamber. The two side walls consisted of 5 cm wide black and white stripes. The shock US (1 s, 0.6 mA) was delivered through the grid floor, which consisted of 3-mm steel rods, spaced 1 cm apart. Background odor was provided by 0.1 ml of a commercially available lavender solution (Airwick Fresh’n’Up) placed in a specimen jar adjacent to the chamber. The conditioning chamber was housed in a light- and sound-attenuating wood cabinet, with illumination provided by a 15W white light and a 15W red light located on the cabinet door. The white noise CS was presented via speakers mounted on the ceiling of the conditioning chamber. The CS was 8 dB louder than the background noise ($\sim$55 dB) produced by ventilation fans.

Testing occurred in a rectangular chamber (13.5 cm long $\times$ 9 cm wide $\times$ 9 cm high) where the front wall, the rear wall, the ceiling, and the floor were made of Plexiglas. The side walls were made of 3-mm steel rods spaced 1 cm apart. No background odor was provided. The test chamber was housed in a light- and sound-attenuating wood cabinet, with illumination provided by a 15W red light located on the cabinet door. The noise CS was delivered via speakers suspended 8 cm from the side walls of the test chamber.

For conditioning, rats were weighed and then placed into the conditioning chamber for 60 s. Three CS–US pairings (separated by a 60-s interstimulus interval) were then given. Each noise CS was presented for 10 s and coterminated with the US. Rats were removed from the conditioning chamber 30 s after the last shock and returned to their home cage.

For test, rats were weighed and then placed into the test chamber for 60 s (pre-CS baseline period), followed by a 120-s presentation of the CS. No shock was given during test. All test sessions were recorded via a camera mounted on the rear wall of the wood cabinet.

Exclusion Criteria

Two criteria were used for the exclusion of data in both experiments. First, rats with a freezing percentage greater than 50% during the baseline period of the test session were excluded from analysis. Three animals from the 24-hr group in Experiment 1 were excluded on the basis of this criterion. Second, rats with a freezing percentage during CS presentation at test that was greater than four standard deviations from the mean group performance were judged as statistical outliers and excluded from analysis. One rat (SD = 8.51 above the mean) from the SAL-1hr group in Experiment 2A was excluded on the basis of this criterion.

Scoring and Data Analysis

The test session was scored for freezing, which was defined as the absence of all movement except that required for respiration (Fanselow, 1980). A time-sampling procedure was used, in which rats were scored as freezing or not freezing every 3 s; this was converted into a percentage of observations scored as freezing. A second scorer unaware of the experimental conditions scored freezing for a random sample of approximately 40% of the rats. The interrater reliability was very high in both experiments ($r_s > .90$). Data was analyzed by either a $t$ test or analysis of variance (ANOVA), as described below. If the ANOVA yielded a significant group effect, then subsequent comparisons were done with the Student-Newman-Keuls (S-N-K) procedure.
Results

Experiment 1: The Kamin Effect

The aim of Experiment 1 was to demonstrate that the Kamin effect occurs in 18-day-old rats when learned fear is indexed by CS-elicted freezing. A three-group, between-subjects design was used; group names refer to the retention interval between conditioning and test sessions. These groups were 1min (n = 10), 1hr (n = 10), and 24hr (n = 8).

Analysis of the level of freezing during the baseline period using ANOVA revealed differences between groups (1min, M = 9.50, SEM = 2.41; 1hr, M = 3.00, SEM = 1.11; 24hr, M = 25.00, SEM = 5.98). This difference was statistically significant, F(2, 25) = 10.54, p < .05. Because of the high levels of baseline freezing in the 24-hr group, we analyzed the CS-elicted freezing levels with an analysis of covariance, with baseline freezing level as the covariate. (An ANOVA using the difference scores yielded the same results.)

Figure 1 shows the mean levels of CS-elicted freezing for rats tested at each of the three retention intervals. The analysis of covariance revealed that there was a significant group difference, F(2, 24) = 20.72, p < .05, and subsequent S-N-K comparisons showed that the level of CS-elicted freezing was significantly lower in rats in the 1hr group than for rats in either the 1min or 24hr groups. These results demonstrate the basic Kamin effect for 18-day-old rats when memory is assessed with CS-elicted freezing.

Experiment 2A: FG7142 and the Kamin Effect

The aims of Experiment 2A were to (a) replicate the Kamin effect observed in Experiment 1, and (b) examine whether a pretest injection of FG7142 alleviates the forgetting observed at the intermediate retention interval. A six-group, between-subjects design was used; the first part of each group label refers to the substance injected 10 min prior to test (i.e., saline [SAL] or FG7142), the second part of each group label refers to the retention interval, and the third part of each group label (if applicable) refers to the dosage of FG7142 administered. The groups tested in this experiment were: SAL-1min (n = 11), SAL-1hr (n = 11), SAL-24hr (n = 11), FG-1hr-1mg/kg (n = 9), FG-1hr-5mg/kg (n = 9), and FG-1hr-10mg/kg (n = 9).

Separate statistical analyses were done to assess each of the experimental aims. The initial analysis included only those rats injected with saline, to determine if the Kamin effect reported in Experiment 1 was replicated. Then the performance of the four groups tested at the 1-hr retention interval was compared to determine if pretest injection of FG7142 alleviated the performance deficit observed after an intermediate retention interval.

Baseline freezing levels were similar in the three groups given a pretest injection of saline, F(2, 30) = 1.78, p = .18 (SAL-1min, M = 5.45, SEM = 2.47; SAL-1hr, M = 1.36, SEM = 0.97; SAL-24hr, M = 10.00, SEM = 4.91). Figure 2 indicates that the level of CS-elicted freezing in rats in the SAL-1hr group was lower than that in rats in either the SAL-1min or the SAL-24hr groups. This description of the data was supported by the statistical analysis, F(2, 30) = 6.05, p = .006. Post hoc S-N-K analysis showed that the CS-elicted freezing in the SAL-1hr group was significantly less than that observed in either of the other two saline-injected groups. Thus, the Kamin effect was once again observed.

The levels of baseline freezing in rats in the four groups tested at the 1-hr retention interval were similar, F(3, 34) = 0.96, p = .42 (SAL-1hr, M = 1.36, SEM = 0.97; FG-1hr-1mg/kg, M = 3.33, SEM = 2.76; FG-1hr-5mg/kg, M = 0.56, SEM = 0.56; FG-1hr-10mg/kg, M = 0.00, SEM = 0.00). CS-elicted freezing was also similar in rats in these four groups, F(3, 34) = 1.19, p = .33. Therefore, an injection of the GABA_A partial inverse agonist FG7142, across three doses, did not attenuate forgetting observed in rats tested at the intermediate retention interval of 1 hr.

Experiment 2B: FG7142 and Infantile Amnesia

This experiment examined whether FG7142 was effective in alleviating infantile amnesia. Although Kim et al. (2006) and Kim and Richardson (2007) previously demonstrated that infantile amnesia is alleviated by FG7142, there were slight variations in experimental procedures, and we wanted to ensure that such variations did not render these procedures insensitive to the effects of FG7142.

A two-group between-subjects design was used: SAL-48hr (n = 11) and FG-48hr-10mg/kg (n = 12). Rats were trained at 16 days of age so that injection of FG7142 or saline occurred when rats were 18 days of age (i.e., rats were the same age at injection in Experiments 2A and 2B). The levels of freezing between the two groups were compared with t tests.

Baseline freezing was similar in the two groups, t(21) = 1.61, p > .05 (SAL-48hr, M = 0.00, SEM = 0.00; FG-48hr-10mg/kg, M = 6.67, SEM = 3.96). Figure 2 indicates that CS-elicted freezing at the 48-hr retention interval was higher in rats in the FG-48hr-10mg/kg group than those in the SAL-48hr group. This difference was statistically significant, t(21) = 2.93, p = .008. Thus, pretest administration of FG7142 alleviated infantile amnesia in rats tested at 18 days of age (see also Kim & Richardson, 2007). This suggests that the failure to observe increased freezing, and therefore improved memory, in rats injected with FG7142 at an intermediate retention interval of 1 hr was not due to a general nonresponsiveness to this agent in 18-day-old rats.

Figure 1. Mean (±SEM) conditioned stimulus elicited freezing percentage as a function of retention interval. The deficit at the 1-hr retention interval defined the Kamin effect.
General Discussion

The present study demonstrates that rats trained at 18 days of age and tested at an intermediate retention interval of 1 hr exhibit less retention, as assessed by CS-elicited freezing, than rats tested at either a 1-min or a 24-hr retention interval. In addition, rats trained at 16 days of age and tested after a 48-hr retention interval also exhibit less retention. The impaired performance at the 1-hr interval is referred to as the Kamin effect, whereas the impaired performance at 48 hr is referred to as infantile amnesia. Pretest injection of the GABA$_A$ receptor partial inverse agonist FG7142 did not alleviate the performance deficit in rats tested at the intermediate retention interval, even when a range of doses were examined, but did when rats were tested at the 48-hr retention interval. Thus, our results suggest that the forgetting defined by the Kamin effect is not due to actions at the GABA$_A$ receptor, whereas that defined by infantile amnesia is. The alleviation of infantile amnesia by pretest administration of FG7142 cannot be attributed to a general effect of this drug on freezing. If FG7142 merely increased freezing levels, then those rats given FG7142 prior to being tested at the intermediate, 1-hr retention interval would have also exhibited an increase in freezing levels; they did not. Further, the differential impact of pretest administration of FG7142 in Experiments 2A and 2B cannot be attributed to different levels of forgetting at these two time points (i.e., the Kamin effect and infantile amnesia), as this was roughly comparable (compare performance of the SAL-1hr and SAL-48hr groups in Figure 2). Rather, our results suggest that forgetting at intermediate and long retention intervals have distinct mechanisms in the developing rat.

This suggestion is consistent with at least one popular view of the Kamin effect. Kamin (1957), and more recently Rudy and Wright-Hardesty (2005) as well as Sutton and Carew (2002), all interpreted the decline in memory observed between the immediate and the intermediate intervals (about 1–6 hr in rats) as caused by temporally discontinuous memory phases. Training initiates the development of at least two memory traces: a short-term and a long-term trace. The Kamin effect occurs because the short-term memory trace has declined before the long-term memory trace has sufficiently formed to support behavioral expression of the memory. In other words, the Kamin effect is caused by a “gap” between short- and long-term memory. Our results further this account by suggesting that, at least in the developing rat, either short-term memory has decayed to an unrecoverable degree or memory inhibition is due to a different mechanism than in long-term memory. Both explanations support the notion that short- and long-term memory are distinct memory systems rather than a single fluctuating memory trace.

The results of the present studies are surprising, however, in the context of another popular account that the Kamin effect reflects a suppression or inhibition of memory retrieval (e.g., Klein & Spear, 1970). As mentioned previously, infantile amnesia is also due to inhibition of memory retrieval, as evidenced by the successful alleviation of forgetting with FG7142 (Kim et al., 2006; Kim & Richardson, 2007). Consequently, one would expect FG7142 to alleviate both the Kamin effect and infantile amnesia; but in our present experiments it did not. It may be important that developing rats were used; the dissociation in physiological mechanisms between the Kamin effect and infantile amnesia, at least in terms of GABA, may be unique to young animals. In other words, just as there is ontogenetic variation in how mammals learn and remember (e.g., Hayne, 2004), so too there may be such variation in how they forget.

Further research is needed to uncover the mechanism mediating the retention failure observed after an intermediate retention interval. If the Kamin effect is due to the decay of a short-term memory trace, then this type of forgetting may not be reversible. From this perspective the only options for alleviating forgetting are either the slowing down of the decay process or the speeding up of the availability of the long-term memory trace. From a different and equally plausible perspective, the Kamin effect may be due to a retrieval failure mediated by a mechanism other than GABA. The identity of this mechanism remains to be elucidated. In any case, uncovering the mechanisms underlying the Kamin effect will contribute not only to our understanding of this specific type of forgetting but also to our understanding of forgetting in general.

References


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