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### Authors

Carvalho, Ofelia M.  
Silva, Alcino J.  
Balleine, Bernard W.

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## **Evidence of Selective Learning Deficits on Tests of Pavlovian and Instrumental Conditioning in $\alpha$ -CaMKII<sup>T286A</sup> Mutant Mice**

**Ofelia M. Carvalho, Alcino J. Silva and Bernard W. Balleine**  
*University of California, Los Angeles, U.S.A.*

Mice carrying a threonine286 to alanine mutation in subunit  $\alpha$  of Ca<sup>2+</sup>/CaM-dependent protein kinase II ( $\alpha$ -CaMKII<sup>T286A</sup>) have been reported to show deficits in hippocampal long-term potentiation as well as spatial learning deficits similar to those induced in mice with hippocampal lesions. In this series, we sought to extend this analysis by investigating the performance of  $\alpha$ -CaMKII mutants on other, potentially hippocampal, learning tasks. Experiment 1 examined fear conditioning, Experiment 2 examined the acquisition of free-operant instrumental conditioning and its dependence upon the action-outcome association, and Experiment 3 assessed interval timing using an instrument version of the peak procedure. We found evidence of a deficit in contextual fear conditioning in Experiment 1 and in peak timing in Experiment 3, but neither a deficit in fear conditioning to a discrete tone CS nor in the acquisition of instrumental conditioning in Experiment 2. Furthermore, all of the mice in Experiment 2 showed a normal instrumental outcome devaluation effect, suggesting that encoding of the action-outcome association was unaffected by this point mutation. It appears, therefore, that although learning in CaMKII mutants is affected on specific tasks, they do not have a general learning deficit, and that the influence of this mutation, and therefore, of  $\alpha$ -CaMKII, is both localized anatomically and relatively specialized functionally.

Recent evidence suggests that, among the signaling molecules involved in the molecular changes that subserve long-term potentiation (LTP),  $\alpha$ CaMKII activation is critical to the way that transient changes in intracellular calcium levels are translated into longer lasting changes in cellular processes. According to one influential model, for example, after the initial binding of calcium and calmodulin, the activated Ca-CaM complex is autophosphorylated at threonine286 (T286), a site adjacent to the calmodulin binding domain, and that in this autophosphorylated state,  $\alpha$ CaMKII can trap calmodulin and remain active even in the absence of calcium influx (Miller & Kennedy, 1986). The ability of  $\alpha$ CaMKII to maintain calcium-independent activity has led to the suggestion that  $\alpha$ CaMKII may serve as part of a memory switch for recent synaptic activity (Lisman, 1994).

In support of this suggestion, it has been reported that inhibition of CaMKII blocks the induction of LTP (Malinow et al., 1989; Silva et al., 1992) and LTP induction both increases CaMKII activity (Fukunaga et al., 1995) and the expression of  $\alpha$ CaMKII in cell bodies 2 h following tetanization and, in proximal and distal dendrites, up to 48 h later (Thomas et al., 1994). Furthermore and consistent with the suggestion that autophosphorylation of  $\alpha$ CaMKII at T286 is critical for

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the induction of LTP, levels of the autophosphorylated form of CaMKII are increased in hippocampal CA1 neurons 30 m following tetanisation (Ouyang et al., 1997) and mice with a point mutation at the T286 site ( $\alpha$ CaMKII<sup>T286A</sup>) show deficient LTP in CA1 (Giese, et al., 1998).

At a functional level, the importance of the role of  $\alpha$ CaMKII in LTP lies in the growing body of evidence that LTP and other forms of synaptic plasticity are required for learning and memory in a number of anatomical areas and using a variety of tasks (Shors & Matzel, 1997; Rison & Stanton, 1996; but see Cain, 1998). In this context, it is of interest that  $\alpha$ CaMKII<sup>T286A</sup> mice have been reported to show selective learning deficits. Thus, for example, T286A mice were found to perform poorly on a spatial but not on a non spatial version of the water maze (Giese et al., 1998), a result that is consistent with reports that CA1 place fields in these mice show reduced spatial selectivity (e.g., Cho et al., 1998). In line with its involvement in LTP, therefore, the autophosphorylation of  $\alpha$ CaMKII at Thr286 appears to be required for normal performance on tasks with a strong hippocampal involvement.

The experiments reported in this paper sought to extend this analysis by investigating the performance of  $\alpha$ CaMKII<sup>T286</sup> mutants on other, potentially hippocampal, learning tasks including contextual fear conditioning (Experiment 1), instrumental conditioning (Experiment 2) and interval timing (Experiment 3).

### **Experiment 1: Fear Conditioning**

Considerable evidence suggests that the hippocampal formation is involved in the learning of conditioned fear to contextual but not to discrete stimuli such as a tone (Fanselow, 2000; Fendt & Fanselow, 1999; Frankland et al., 1998) and several studies have implicated CaMKII activation in context fear. Thus, for example, the injection of pharmacological inhibitors of CaMKII into the hippocampus has been reported to block learning on an inhibitory avoidance task (Wolfman et al., 1994). Furthermore, contextual fear conditioning is both deficient in  $\alpha$ CaMKII knockout mice (Chen et al., 1993; Silva et al., 1996) and results in an elevation in levels of autophosphorylated CaMKII in the hippocampus (Atkins et al., 1998). These findings strongly predict that mice with a point mutation at the T286 site will show a deficit in contextual fear conditioning but not in learning about discrete cues. We tested this prediction in the current experiment.

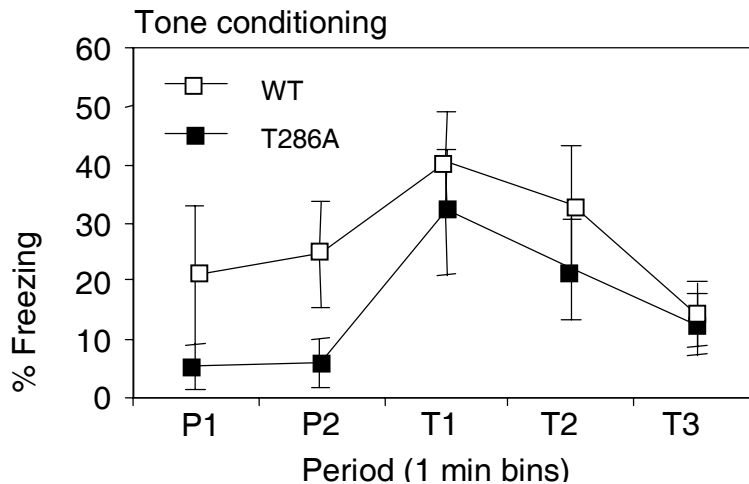
### ***Methods***

***Subjects and Apparatus.*** The subjects were 15, C57/BL/6 mice comprising 8 homozygous  $\alpha$ CaMKII<sup>T286A</sup> mutants generated using the Pointlox procedure as previously described (Giese et al., 1998) and backcrossed into a C57/BL/6 background from the original 129sv source, and 7 wild type (WT) littermates. The mice were group housed in 3 squads of four and one squad of three in the Department of Psychology vivarium and maintained on a 12-hour light-dark cycle with lights on at 06:00 h, with food and water freely available. All experimental procedures were conducted in the light phase of the cycle. Mice were trained in conditioning chambers (MED Associates, Inc., Vermont, U.S.A.) that had a stainless steel grid floor through which a 2-s, 0.75-mA footshock could be delivered. The boxes were also equipped with a tone generator that could deliver a 2800-Hz, 85-dB tone stimulus. Testing took place in the training boxes (Room A - context test) and in a different set of boxes that differed in physical and olfactory properties and were positioned in a separate room (Room B - tone test). Our index of conditioning was the freezing response that was measured using an automated procedure as previously described (Anagnostaras et al., 2000).

**Procedure.** Fear conditioning was conducted following procedures previously described (Frankland et al., 1998). Briefly, on the training day the mice were placed in the conditioning chambers in Room A and, after 2 min had elapsed, a 30 s tone was presented the last 2 s of which overlapped with the footshock. Three tone-shock pairings were presented in this manner, each separated by a 30-s intertrial interval. After an additional 30 s the mice were returned to their home cage. The day after this training session the mice were placed once again in the conditioning boxes in Room A and the levels of freezing were scored for a 5 min period in the absence of the tone (context test). The next day the mice were placed in the test chambers in room B and tone-dependent freezing was assessed. Two minutes after placement in the test chamber, the 2800-Hz, 85-dB tone used in conditioning was presented for 3 min. Freezing was scored during the entire 5 min of both the context and tone tests using a time sampling method as previously described (Frankland et al., 1998)

## Results and Discussion.

The results of the tone and context tests are presented in Figures 1 and 2 for each minute of the 5-min tone (Figure 1) and context (Figure 2) test periods. From these figures it appears that, whereas freezing in the initial two min period of the tone test (i.e., P1 and P2) differed between the mutant and WT animals, freezing recruited by the tone itself (i.e., T1, T2, and T3) did not appear to differ between groups. It is possible that the difference in the pretone period in this test reflected different degrees of generalization of conditioned fear to the contextual cues present during training and, in support of this suggestion, a clear difference between the groups in freezing to contextual stimuli was observed in the context test. Therefore, the point mutation at T286 produced a striking and selective deficit in learning to associate shock with contextual cues but not with a discrete tone CS.



**Figure 1.** Freezing during the tone test in Experiment 1 as a percentage of total behavior in wild type (WT) and T286A mutant mice ( $\pm 1$  standard error of the mean). Freezing in the 2-min prior to tone presentation is presented at points P1 and P2 and during the 3-min of tone presentation at points T1, T2 and T3.

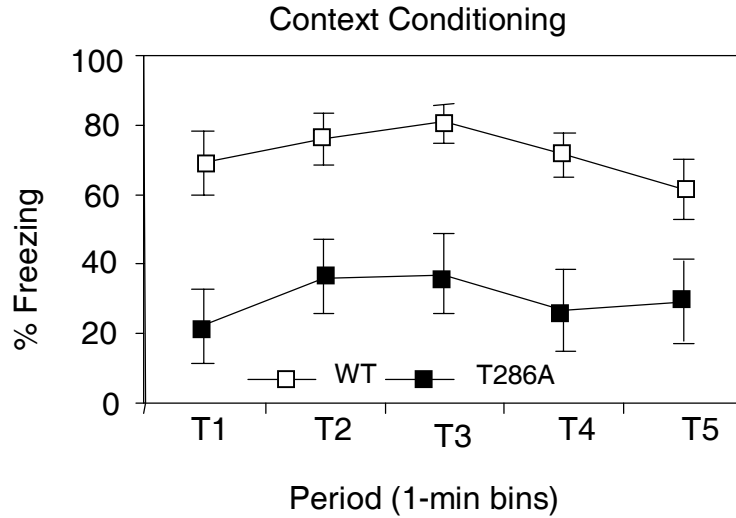


Figure 2. Freezing during the context test in Experiment 1 as a percentage of total behavior in wild type (WT) and T286A mutant mice ( $\pm 1$  standard error of the mean). Freezing is presented divided into 1-min bins, i.e. for each minute of the 5-min test.

This description of the data was supported by the statistical analyses. A two-way mixed analysis of variance (ANOVA) was conducted on the tone test using a between-subjects factor of Group, separating freezing in mutants and WT, and a within-subjects factor of Stimulus, separating freezing prior to the tone from that observed during the tone presentation. (alpha was set at 0.05 in all statistical tests). This analysis revealed no effect of Group,  $F(1, 13) = 1.80$ , but found a significant effect of Stimulus,  $F(1, 13) = 23.10$ , and a significant interaction between these factors,  $F(1, 13) = 4.20$ . To confirm the apparent group difference before tone presentation, simple effects analyses were conducted on the significant interaction. These analyses revealed a significant effect of group in the pretone period,  $F(1, 13) = 8.00$ , but no difference between groups during the tone,  $F < 1$ . For the context test, a comparable analysis was conducted using factors Group and Period (five 1-min bins on test). This analysis revealed a significant effect of group,  $F(1, 13) = 11.40$ , but neither an effect of period,  $F(1, 13) = 1.60$ , nor a reliable Group x Period interaction,  $F < 1$ .

The results of this experiment support the prediction derived above: Mice with a point mutation of the T286 site ( $\alpha\text{CaMKII}^{\text{T286}}$ ) were found to have a clear deficit in contextual fear conditioning as assessed on the context test but showed conditioned freezing to a tone stimulus that did not differ from WT controls. Although freezing to the tone was numerically less than that observed in the WT, this difference was not statistically reliable and, if anything, the mutant mice showed a greater increase in freezing during the tone relative to freezing in the pretone period than WT. Indeed, this difference in pretone freezing is, itself, consistent with a selective deficit in contextual conditioning in the mutants. To the extent that generalization between contexts occurred, a degree of freezing to the novel (Room B) context might have been anticipated and the failure to observe such generalization in the mutants suggests that the context exerted much weaker conditional control over freezing than in the WT. As such, we can conclude that autophosphorylation

at the T286 site is important for contextual conditioning and, to the extent that this conditioning involves the hippocampal formation, that the deficit in  $\alpha$ CaMKII activation was relatively selective to the hippocampus in these mice.

### **Experiment 2: Instrumental Conditioning**

The ability of a hungry rat to acquire seemingly arbitrary actions, such as lever pressing, to gain access to food is one of the most robust forms of learning one can observe in the animal laboratory. Significant recent advances have been made in our understanding of the psychological determinants of instrumental action in the rat. For example, outcome devaluation studies have provided clear evidence that, in instrumental conditioning, rats are able to encode the specific consequences of their actions and that the encoded action-outcome (A-O) relation plays a critical role in the initial acquisition and performance of an instrumental action (see Balleine, 2001; Dickinson & Balleine, 1994; Colwill & Rescorla, 1986, for detailed reviews of this issue). When overtrained, however, instrumental performance can become stimulus bound, independent of the current value of the outcome and so impervious to outcome devaluation (Adams, 1982; Dickinson & Balleine, 1995) suggesting the increasing involvement of a stimulus-response (S-R) process as performance becomes more habitual.

This functional distinction between the A-O and S-R learning processes has often been interpreted as implying that different memory systems contribute to instrumental conditioning and a number of authors have suggested that A-O associations are encoded in declarative memory whereas S-R associations are encoded in procedural memory (Dickinson, 1980; Dickinson & Balleine, 1993; Squire & Zola-Morgan, 1996; Winograd, 1975). In the current context, it is important to note that considerable recent evidence suggests that declarative memory is dependent upon the integrity of the hippocampal formation (Eichenbaum et al., 1996; Squire, 1992; Squire & Zola-Morgan, 1996) implying that instrumental learning may be hippocampally dependent. This hypothesis predicts that damage to the hippocampus should render animals unable to encode the relation between actions and their outcomes with the effect that their instrumental performance should be controlled predominantly by the S-R process and, therefore, relatively insensitive to outcome devaluation. This prediction was assessed in Experiment 2 using the  $\alpha$ CaMKII<sup>T286</sup> from the same genetic stock as those used in Experiment 1.

Mice were food deprived and trained to press a lever for food pellets on a continuous reinforcement schedule until they had earned 100 pellets. We then assessed the integrity of their A-O learning using a specific-satiety outcome devaluation procedure (cf. Balleine & Dickinson, 1998). To achieve this, mice were given two tests on the levers in extinction, one following satiety on the training food pellets and a second after satiety on a novel sucrose solution. We anticipated that wild type mice would show a selective outcome devaluation effect, that is, a greater reduction in performance after satiety on the pellets than after satiety on the sucrose. If T286 mutants are unable to encode the specific consequences of their actions, however, we anticipated that they would show a deficit in this selective devaluation effect, with satiety on pellets and sucrose having a similar effect on subsequent lever press performance.

## **Methods**

**Subjects and Apparatus.** The subjects were 8 WT and 9 homozygous (T286A) experimentally naïve mice from the same  $\alpha$ -CaMKII $\beta$ T286A population as those used in Experiment 1 and housed under similar conditions (two squads of four and three squads of three). Water was available in the home cage but all mice were 22-h food deprived during all of the behavioral procedures. The mice were trained in eight operant chambers (MED Associates) each containing a retractable lever, a pellet dispenser, that could deliver 20 mg Noyes food pellets, and a dipper that delivered 0.02 ml of a 10% sucrose solution. The mice were also given sessions in which they were allowed to freely consume the food pellets or the sucrose solution and, for these sessions, each mouse was placed in a box identical to its home cage equipped with either a water bottle containing the 10% sucrose solution or a small dish containing 5 g of food pellets.

**Procedures.** All animals received three sessions of magazine training with the Noyes food pellets in the operant chambers with the lever retracted. In each session, the mice were placed for 30 min in an operant conditioning box and the pellets being delivered at random times with a programmed average of one pellet each 60 s (RT-60). Immediately following each magazine training session the mice were acclimated to the consumption boxes for 30 min and in each session received 10 pellets in the food dishes and 5 min access to a 10% sucrose solution.

The day after the third magazine training session, the mice were trained to lever press. In these sessions, the lever was introduced into the chamber and each press delivered one Noyes pellet into the recessed magazine. This training period continued until each mouse had earned 100 pellets or until 1 h had elapsed.

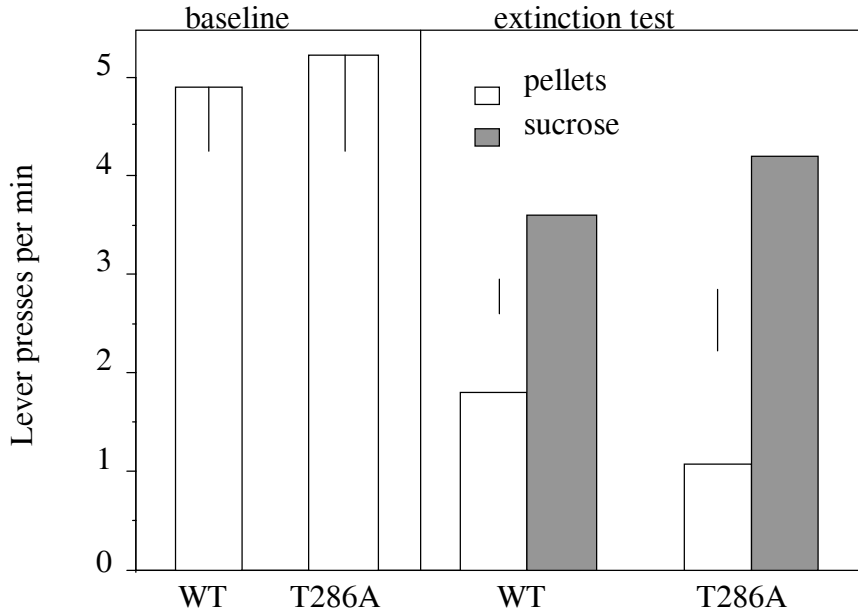
**Devaluation Test.** The day after the final instrumental training session, the first devaluation test was conducted. Before this test, 4 wild type and 5 mutant mice were placed in the feeding cages and were allowed access to the Noyes pellets for 1 h. The remaining animals were placed in the cages and allowed to drink the 10% sucrose solution during this period. Immediately following this exposure, lever pressing in both groups was assessed in a 10-min extinction test in which the lever was presented but no reward was delivered. The following day the groups were reversed; mice given pellets to eat during the first devaluation session were now given 10% sucrose for 1 h whereas those previously given sucrose were given the pellets for 1 h immediately after which a second 10-min extinction test was conducted exactly as the first.

## **Results and Discussion**

All of the mice acquired the lever press response for the food pellet reward. Furthermore, there was no difference between the wild type (mean: 1.75 sessions; range 1-3) and the mutant mice (mean: 1.88 sessions; range 1-3) in the rate at which they acquired this response. The results of the devaluation extinction test are presented in Figure 3. It is clear from this figure that both the WT and T286A mice performed less presses when fed pellets (training outcome) than when fed sucrose with the difference in performance being, if anything, greater in the mutant than in the WT group. The statistical analysis confirmed this observation. A 2-way ANOVA conducted with a between-subjects factor of Group and a within-subjects factor of Devaluation, separating performance on the lever after satiety on sucrose and pellets, yielded a significant effect of Devaluation,  $F(1, 15) = 15.60$ , but neither an effect of Group,  $F(1, 15) = 1.70$ , nor a Group x Devaluation interaction,  $F(1, 15) = 2.20$ .

The results of Experiment 2 suggest that instrumental learning was not affected by the point mutation at the T286 site. Thus, relative to wild type controls, the T286A mutants acquired lever pressing for food at a comparable rate. Furthermore, no evidence was found suggestive of a deficit in the acquisition of the A-O association in the T286A mice, and the difference in performance when sated on

the training outcome relative to satiety on a different outcome was, if anything, larger in the mutants than in the wild types. Thus, we have good evidence that the T286A mutants were able to encode the specific consequences of their actions and, when the incentive value of those consequences was changed, to use that encoding to modify their performance on test in a selective manner.



**Figure 3.** Mean lever presses per min during training ( $\pm 1$  standard error of the mean; left panel) and during the outcome devaluation extinction tests ( $\pm 1$  standard error of the difference of the means; right panel) conducted in Experiment 2. Data are presented for wild type (WT) and T286A mutant mice and, for the extinction tests, divided into performance on the test before which the mice were satiated on the training outcome (pellets) and on a different nutritive outcome (sucrose).

### Experiment 3: Interval Timing

The evidence for the involvement of the hippocampal formation in instrumental conditioning was largely derived from conjecture based on evidence from studies of humans with specific deficits in declarative memory and so it is, perhaps, not surprising that the T286A mutants were not impaired on this learning task. There is, however, quite good evidence that the hippocampus plays a role in the way animals time the performance of their instrumental actions when reward is delivered on a fixed interval schedule. For example, Meck et al. (1984) found evidence that the clock speed in rats with the fimbria-fornix transected was faster than in sham controls such that the fornix rats routinely responded earlier in the fixed interval than the sham controls. As such, we decided to assess fixed interval timing in T286A mutants using the standard peak timing procedure that has been used extensively in rats to assess fixed interval timing (e.g. Hinton & Meck, 1997; Church et al., 1994).



## Methods

**Subjects and Apparatus.** The same mice used in Experiment 2 were used in this experiment. The mice were maintained as described for Experiment 2 and were trained in the same operant chambers.

**Procedure.** The mice had already been trained to lever press on a continuous reinforcement schedule (see Experiment 2). On each of the three days after the second devaluation test in Experiment 2, the mice were trained on a fixed interval 40 s (FI-40) schedule of reinforcement with the levers continuously extended. Thereafter, each trial was signaled by the extension of the lever and ended with its retraction, either after the reward had been earned by the first response after the 40-s period had timed out or, on peak test trials, after 80 s had elapsed. The rewarded and peak test (non-rewarded) trials were intermixed in each session with 40 rewarded trials and 10 nonrewarded trials presented with a variable 45-s intertrial interval. On test trials, the lever was extended and retracted after a multiple of the training target interval was met; no food was available on these trials. For the first 30 sessions, the criterion reward interval was 40 s. For the next 30 sessions the criterion interval was changed to 20 s.

The experiment was controlled through a MED Associates interface connected to a PC-compatible computer running a MED-PC software system. Responses were recorded in real time. Data recorded during both rewarded and nonrewarded trials were used in the analyses. Additional programs were used to extract the mean response rates and individual peak times on the non-rewarded trials, which were then subjected to further statistic analysis as described below.

**Data analysis.** Data from the last 4 sessions of nonrewarded peak trials were used to estimate the response peak time, peak rate, and precision of timing for each subject. The number of responses (in 4-s bins) was averaged over sessions to obtain a mean response rate for each subject. Subsequent analyses used the mean response rate function in an interval centered on the criterion time (20-s and 40-s) and twice as large as the criterion (40-s and 80-s). The response rate function in this interval was fitted using the Marquardt-Levenberg iterative algorithm to find the coefficients (parameters) of the model that give the “best fit” (square root minimization) between the equation and the data. The following generalized Gaussian+linear model was fit to the individual mean response rate:

$$R(t) = a * \exp(-.5 * ((t-t_0)/b)^2) + c * (t-t_0) + d$$

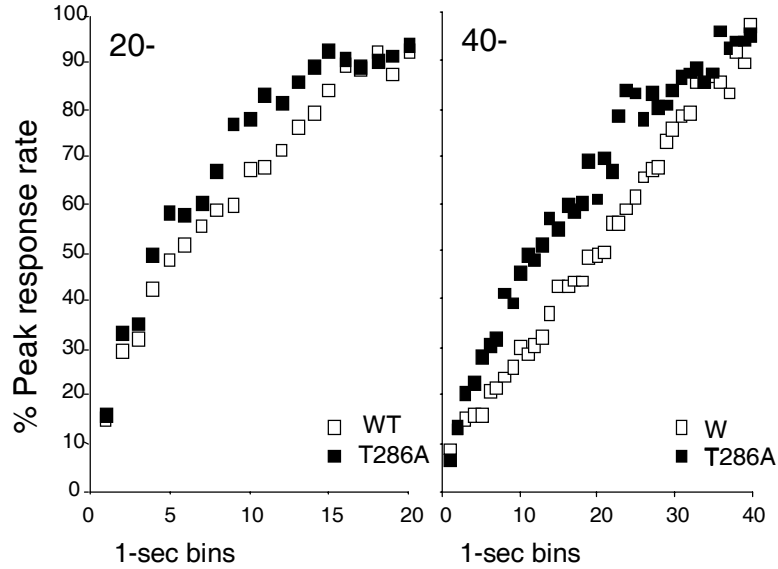
where  $t$  is the current time and  $R(t)$  is the mean number of responses at time  $t$ . The iterative algorithm provided parameters  $a$ ,  $b$ ,  $c$ ,  $d$  and  $t_0$ . Parameter  $t_0$  was used as an estimate of the response peak time,  $a+d$  was used as an estimate of the peak rate of response, and parameter  $b$  was used as an estimate of the precision of timing. Parameter  $c$  was used to evaluate the linear increase in response during the presence of the stimulus. An estimate of the coefficient of variation (COV) was obtained by dividing parameter  $b$  by parameter  $t_0$ . Estimates of parameters  $t_0$ ,  $a+d$ ,  $b$ ,  $c$ , and COV were submitted to repeated measures ANOVAs with factors Group (WT vs. T286) and Criterion (20-s vs. 40-s).

Because of the inherent differences in response rate between mice, when averaging data over mice the peak in the mean response rate tends to be influenced by mice with a higher response rate. Therefore, a mean percent response rate was computed using data collapsed over sessions. A maximum response rate was computed for each mouse, and the individual percent maximum response rate functions were averaged over mice. All statistical tests were conducted on these transformed data.

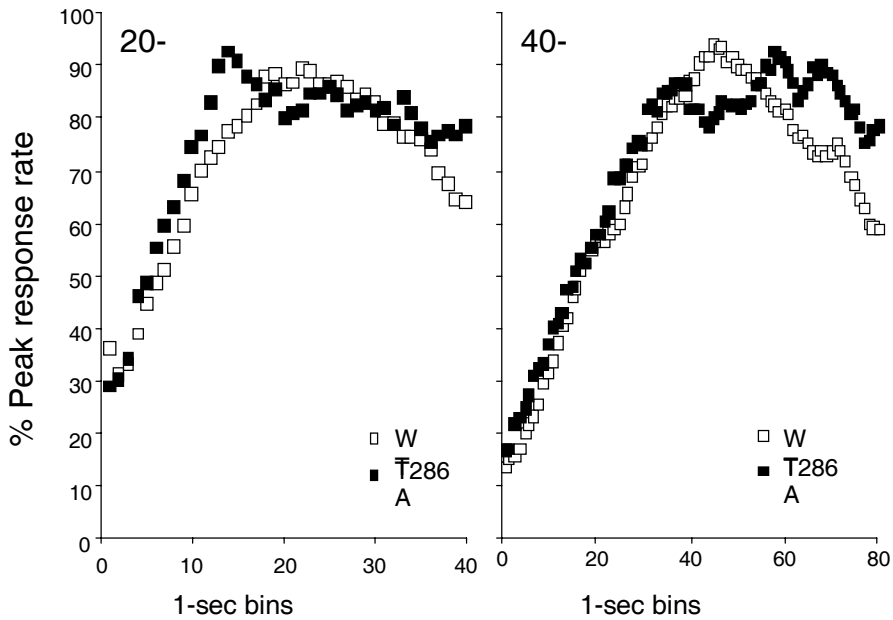
## Results and Discussion

The average response rates during rewarded trials are shown in Figure 4 and in Figure 5 for the nonrewarded peak trials for both the 20-s and 40-s criterion times. In both groups, at both criterion times and during both rewarded and nonrewarded trials, response rate increased over the duration of the fixed interval preceding reward. On rewarded trials it appears that the T286A mice responded at a higher rate early in the FI and on both criterion times suggesting that clock time in these mice was significantly faster than in the WT group. This appeared also to be true of peak

trials, but, in addition, there was some suggestion that the T286A mice continued to maintain performance after the criterion time had elapsed. Hence, the pattern of results appears to suggest that the T286A mice showed less precision in their timing of reward delivery than the mice in Group WT.



**Figure 4.** Mean responding in wild type (WT) and T286A mutants on reinforced trials in 1-s bins as a percentage of the highest (peak) response rate during the interval. in 1-s bins as a percentage of the peak response rate during the interval and averaged over 4-s bins.



**Figure 5.** Mean responding in wild type (WT) and T286A mutants on non-reinforced (peak) trials. Data are presented in 1-s bins as a percentage of the peak response rate during the interval and averaged over 4-s bins.

This description of the data was largely supported by the statistical analysis. A repeated measures ANOVA (Group x Criterion) of the estimated response peak time (parameter  $t_0$ ) showed a significant effect of Criterion,  $F(1, 17) = 122.70$ , and Group,  $F(1, 17) = 5.34$ , but no interaction. Response peak time peaked significantly faster in the T286A group than in the WT group. In the WT mice, the response rate peak time was not significantly different from the criterion time at both timing criteria. In WT subjects, response rate peaked at 21.38 s for the 20-s criterion [ $t(8) = 0.78$ ], and at 44.8 s for the 40-s criterion [ $t(8) = 1.86$ ]. In the T286A subjects, the response rate peaked significantly faster for the 20-s criterion (mean peak time 14.91-s,  $t(9) = 2.32$ ) whereas, at the 40-s criterion, although the response rate peaked faster in T286A mice (mean peak time 37.64 s) than in WT mice, this difference failed to reach significance,  $t(9) = 0.65$ .

A repeated measures ANOVA (Group x Criterion) of the precision of timing (parameter  $b$ ) showed a significant effect of Criterion,  $F(1, 17) = 38.49$ , but no other effects or interactions. The response was significantly more precise (smaller width of the response function, parameter  $b$ ) for the 20-s criterion than for the 40-s criterion. To evaluate whether the precision of timing was proportional to the timing criterion (scalar property) for each subject we computed a coefficient of variation COV by dividing the precision (parameter  $b$ ) by the peak time (parameter  $t_0$ ). A repeated measures ANOVA (Group x Criterion) of the COV showed a significant effect of Group,  $F(1, 17) = 5.17$ , but no other effects or interactions. The lack of a significant effect of Criterion suggests that in both groups the response follows the scalar property (i.e., constant COV at different timing criteria). Even so, the T286A mice showed a significantly larger COV (mean COV = 0.9) than the WT mice (mean COV = 0.54) suggesting a decreased precision of timing in T286A subjects relative to WT subjects at both criterion times.

Finally, as shown in Figure 5, mice in both groups continued to show a relatively large response even toward the end of the test period (i.e., at the end of the 2nd FI). By fitting a generalized Gaussian+linear model to the individual mean response rate, we separated the contributions of the timing of the stimulus (Gaussian) and of the simple presence of the lever stimulus (linear). A repeated measures ANOVA (Group x Criterion) of the linear component (parameter  $c$ ) failed to suggest significant effects or interactions, supporting the notion that the presence of the linear component was similar in both groups, at both timing criteria. In line with this suggestion, a repeated measures ANOVA (Group x Criterion) of the response peak rate (parameter  $a+d$ ) showed a significant effect of Criterion,  $F(1, 17) = 14.59$ , but no other effects or interactions. The response rate was significantly higher for the 40-s criterion (37.25 responses/min) than for the 20-s criterion (21.65 responses/min), suggesting that the increased response rate for the 40-s criterion might be due to the increased duration of the linear component for the longer duration of the 80-s test. Nevertheless, the fact that overall response rate failed to show differences between WT and T286A subjects suggests that the results cannot be simply due to motor disturbances in T286A mice.

In summary, the results of Experiment 3 suggest that both WT and T286A mice are able to time, and that their responding obeys the scalar property of timing, a fundamental property of timing that has been demonstrated in mice and other species (Gibbon et al., 1984; Lejeune & Wearden, 1991; Roberts & Boisvert, 1998;

Brodbeck et al., 1998). Furthermore, an analysis of the response peak time suggests an increase in the speed of an internal clock in the T286A mice relative to WT mice, a suggestion supported by the fact that in both groups the COV was found to be equal at both criteria. Nevertheless, the COV was found to be greater in T286A mice than in WT mice, suggesting a decrease in the precision of timing in T286A mice. This is of interest because, in currently influential models of timing, such as scalar expectancy theory (e.g. Church et al., 1992; 1994; Gibbon, 1977), timing relies on comparing the current time stored in an accumulator with a sample from memory. According to this theory, the decrease in the precision of timing may be accounted for by an increase in the variability of sampling from memory. As such, the timing deficit observed in the T286A mutants can be argued to be compatible with both increased clock speed and memory sampling accounts and further experimentation will be required to separate these two explanations of the current data.

### **General Discussion**

The current experiments provide evidence of selective learning deficits in mice with a point mutation at Thr286 of  $\alpha$ CaMKII. In Experiment 1, a deficit was found in conditioned freezing elicited by the context but not by a discrete tone cue in T286A mice. Furthermore, in Experiment 3, T286A mice appeared to be deficient in their ability to time the performance of an instrumental lever press response based on a fixed interval of reward delivery. Nevertheless, in Experiment 2, T286A were not impaired in their acquisition of instrumental lever pressing nor were they impaired in their ability to encode the consequences of lever pressing and showed normal sensitivity to a posttraining change in the value of the instrumental outcome. Thus, in the Pavlovian and instrumental conditioning tasks assessed in this series, the evidence consistently suggests that autophosphorylation of  $\alpha$ CaMKII at Thr286 is selectively involved in the learning of certain aspects of these tasks but not others.

One reason for the selective involvement of  $\alpha$ CaMKII in the current experiments may be that this point mutation is relatively localized anatomically. Previous studies found evidence of deficits on a spatial but not on a non-spatial version of the water maze in T286A mice similar to deficits induced by hippocampal lesions, raising the possibility that deficits in these mice are limited to hippocampal tasks (cf. Giese et al., 1998). In support of this suggestion, deficits in contextual fear conditioning (Fanselow, 2000) and interval timing (Meck et al., 1984) have both been reported in rats with hippocampal damage that are similar to the deficits observed in the current experiments. Furthermore, a recent investigation of the involvement of the hippocampus in instrumental conditioning found that electrolytic lesions of dorsal hippocampus failed to influence either instrumental acquisition or outcome devaluation effects (Corbit & Balleine, 2000). These lesions did however affect the sensitivity of instrumental performance to a reduction in the instrumental, A-O contingency raising the possibility that the same effect might be found in T286A mutant mice, a prediction that we will be interested to assess in future experiments. Nevertheless, it is important to note that, although these procedures all depend on the hippocampus, it is possible that they depend on different biochemical mechanisms within that structure. As such, the present data are important in

suggesting that the autophosphorylation of this kinase is an essential step in the ability of the hippocampus to support a wide variety of learning tasks.

In addition to the selective deficits observed in the T286A mutants, it is worth pointing out that the current experiments, particularly Experiment 2, provides new information about the performance of WT mice on learning tasks. To date there has been very little basic behavioral research on mice, certainly much less than has been conducted using rats as subjects, and this is clearly something that must be overcome if the genetic bases of learning are to be fully characterized. To this end, Experiment 2 represents the first reported finding that, in instrumental conditioning, mice, like rats, encode the consequences of their actions and that the performance of their actions is sensitive to the current incentive value of the instrumental outcome (cf. Balleine, 2001). Furthermore, the results of Experiment 3 provide an important additional demonstration to the literature documenting that mice are able to time intervals of reinforcement (see also Lejeune & Wearden, 1991; Brunner & Hen; 1997; King et al., 2001; Meck, 2001; Abner et al., 2001) and in a manner similar to that observed in rats and other species (e.g., Brodbeck et al., 1998; Church et al., 1994; Meck et al., 1984). Thus, in expanding the range of tasks on which the behavior of mice has been assessed and in establishing the selective involvement of  $\alpha$ CaMKII in those tasks, we believe that the current experiments make a significant contribution to research investigating the genetic bases of behavior.

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