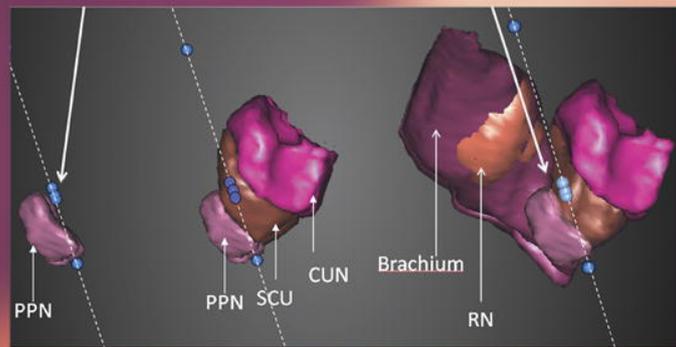


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## CALCINEURIN INHIBITION ELIMINATES THE NORMAL INVERTED U CURVE, ENHANCES ACQUISITION AND PROLONGS MEMORY IN A MAMMALIAN 3'-5'-CYCLIC AMP-DEPENDENT LEARNING PARADIGM

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**Abstract**—The role protein phosphatase 2B (calcineurin, CaN) plays in learning and memory has received a significant amount of attention due to its promotion of the dephosphorylation of 3'-5'-cyclic AMP response element binding protein (CREB). Researchers have ascertained that overexpression of CaN is associated with memory retention deficits [Foster TC, Sharrow KM, Masse JR, Norris CM, Kumar A (2001) Calcineurin links Ca<sup>2+</sup> dysregulation with brain aging. *J Neurosci* 21:4066–4073; Mansuy IM, Mayford M, Jacob B, Kandel ER, Bach ME (1998) Restricted and regulated overexpression reveals calcineurin as a key component in the transition from short-term to long-term memory. *Cell* 92:39–49], while CaN inhibition enhances learning and memory [Gerdjikov TV, Beninger RJ (2005) Differential effects of calcineurin inhibition and protein kinase A activation on nucleus accumbens amphetamine-produced conditioned place preference in rats. *Eur J Neurosci* 22:697–705; Ikegami S, Inokuchi K (2000) Antisense DNA against calcineurin facilitates memory in contextual fear conditioning by lowering the threshold for hippocampal long-term potentiation induction. *Neuroscience* 98:637–646]. The present study hypothesized that infusion of a CaN inhibitor (FK506) bilaterally into the olfactory bulbs of postnatal day 6 Sprague Dawley rat pups would prolong the duration of a conditioned odor preference and retard cyclic AMP response element binding protein dephosphorylation. A 2 mg/kg s.c. injection of isoproterenol (ISO,  $\beta$ -adrenoceptor agonist) was paired with a 10 min exposure to peppermint and subsequently an infusion of FK506. Immunohistochemistry for phosphorylated 3'-5'-cyclic AMP response element binding protein (pCREB) revealed that unilateral infusion of FK506 resulted in an amplification of phosphorylated CREB in the olfactory bulb 40 min after training compared with saline-infused bulbs. Pups infused bilaterally with FK506 maintained a learned preference for peppermint 48, 72 and 96 h after training. CaN inhibition also modified the conventional inverted U curve obtained when ISO is used to replace stroking, as the unconditioned stimulus. When pups were infused with FK506, learning occurred with sub- and supra-optimal doses of ISO indicating

that CaN overcomes non-optimal effects ISO may have on learning. We demonstrate that CaN inhibition can extend the duration of conditioned olfactory memory and may provide a target for memory prolongation that is superior to even phosphodiesterase inhibition observed in previous studies. © 2009 Published by Elsevier Ltd on behalf of IBRO.

**Key words:** protein phosphatase 2B, FK506, odor preference learning, rat pups, isoproterenol.

For nearly two decades theoreticians and experimental biologists have recognized that memory suppression proteins should have equally important, if opposite, roles in memory acquisition and storage to those of memory promotion (Lisman, 1989; Wang and Kelly, 1997; Abel and Kandel, 1998). The importance of kinases in memory promotion has long been recognized, while the roles of phosphatases in memory suppression are now being explored. The calcium-dependent phosphatase, calcineurin (CaN), composes 1% of brain protein (Yakel, 1997) (for review) and is co-localized with protein kinase A at postsynaptic densities through the synaptic anchoring protein, AKAP79 (Coghlan et al., 1995; Yakel, 1997). Based on this co-localization CaN would be predicted to have a key role in the modulation of 3'-5'-cyclic AMP (cAMP)-dependent learning and memory.

The cAMP cascade has been implicated in a variety of learning and memory models, from invertebrates (Castellucci et al., 1980; Bailey et al., 1996) to vertebrates (Thompson et al., 2000), but, in mammals, while implicated in numerous paradigms, the cAMP cascade has only been shown to be both necessary, and *sufficient*, for odor preference learning in the neonate rat (McLean et al., 2005).

Infusion of a  $\beta$ -adrenoceptor antagonist in the olfactory bulb prevents odor preference learning (Sullivan et al., 1992), while infusion of a  $\beta$ -adrenoceptor agonist paired with odor (Sullivan et al., 2000), induces odor preference learning. Preventing cAMP breakdown extends odor preference memory (McLean et al., 2005) consistent with a causal role for cAMP. Successful odor preference learning is also associated with a temporally specific pattern of cAMP increase and decrease (Cui et al., 2007).

A single 10 min odor preference training trial produces a 24 h odor preference memory that is protein synthesis dependent (McLean, unpublished observations). Multiple training trials spaced over days can produce a lifetime memory (Coopersmith and Leon, 1986; Shah et al., 2002). Phosphodiesterase (PDE) inhibition can increase one-trial 24 h memory duration to 48 h and longer (McLean et al.,

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**Abbreviations:** ANOVA, analysis of variance; cAMP, 3'-5'-cyclic AMP; CaN, calcineurin, protein phosphatase 2B; CREB, 3'-5'-cyclic AMP response element binding protein; CS, conditioning stimulus; DMSO, dimethyl sulfoxide; ISO, isoproterenol,  $\beta$ -adrenoceptor agonist; pCREB, phosphorylated 3'-5'-cyclic AMP response element binding protein; PDE, phosphodiesterase; PND, postnatal day; UCS, unconditioned stimulus.

2005). This result suggests cAMP cascade events may control a continuum of 'long-term' memory durations. Inhibition of CaN, which reduces dephosphorylation (Lin et al., 2003; Snyder et al., 2003; Yang et al., 2004), is also associated with PDE inhibition (Rusnak and Mertz, 2000), and promotes activation of adenylyl cyclase IX that is highly expressed in the olfactory bulb and other memory areas (Antoni et al., 1998). These signaling cascades should increase odor preference memory duration in the one trial model.

Yuan et al. (2003b) also hypothesized that interactions between phosphorylating events via PKA and dephosphorylating events via CaN in olfactory bulb mitral cells could account for the inverted U curve relationship of unconditioned stimulus (UCS) activation to optimal acquisition in the odor preference paradigm. Dephosphorylation induced by high levels of  $\beta$ -adrenoceptor agonist was proposed to favor memory suppression over memory promotion. Parallel initiation of kinase and phosphatase actions might also set the threshold for the UCS level required for initial learning.

CaN is the primary candidate to provide yin/yang control of cAMP-initiated functional events in mitral cells. The present experiments test this idea. Local infusion of the CaN inhibitor, FK506, is used to assess the contribution of olfactory bulb CaN activity to cAMP-dependent odor preference memory and learning in the rat pup. The results are consistent with a role for CaN in controlling both the expression and acquisition of the odor preference memory trace.

## EXPERIMENTAL PROCEDURES

### Animals

A total of 252 Sprague–Dawley rat pups were used from 34 litters culled to 12 pups on postnatal day (PND) 0 or 1. All dams were housed in polycarbonate cages containing hardwood chips on a 12-h light/dark cycle at 21 °C in the animal care facility at the Health Sciences Centre of Memorial University of Newfoundland. Prolab RMH 3000 rat diet (Purina Mills, Richmond, IN) and water were available *ad libitum*. All procedures were approved by the Memorial University Institutional Animal Care Committee and conformed to the standards set by the Canadian Council on Animal Care. Effort was made to minimize the number of animals used and their suffering.

### Cannulae assembly

CaN inhibition was performed via direct intrabulbar infusion of FK506. Two guide cannulae (Small Parts Inc., FL, USA, 23 gauge tubing cut to 6 mm), anchored in dental acrylic, were 2 mm apart and extended 1 mm beyond the acrylic. Insect pins were placed into each guide cannula to prevent blood clots in the cannulae. Infusion cannulae were made of 30 gauge stainless steel tubing cut to a length of 10 mm. One end was inserted into PE20 polypropylene tubing. Each attached infusion cannula and tubing extended 1 mm below the 23 gauge guide cannula.

### Surgery

On PND 5, each pup was anesthetized by hypothermia and secured in a modified stereotaxic holder. The skull was exposed and the cannula assembly was attached to the skull over holes drilled above the olfactory bulbs. Dental acrylic secured the assembly to the skull. The cannula area was sutured together and covered with Bitter Orange (Gourmet Pet, GA, USA). Following surgery, the

pups were warmed on a heating pad and returned to the dam when they were alert.

### Olfactory learning, drug injection, and sample collection

On PND 6, each pup was removed from the dam, given an s.c. injection of isoproterenol (ISO, Sigma Chem.) or saline and placed back with the dam for 30 min. Pups were placed on clean bedding 10 min prior to training. Subsequently, each pup was placed on peppermint scented bedding (conditioning stimulus, CS) for 10 min (0.3 ml of peppermint extract per 500 ml of bedding). Immediately following training each pup received 1 min bilateral intrabulbar 1  $\mu$ l infusion of vehicle (100% dimethyl sulfoxide, DMSO) or 5, 10 or 20 mM FK506 (CaN inhibitor Alexis Biochemicals, Switzerland) dissolved in vehicle. The use of DMSO as vehicle and concentration range of infused FK506 was based on previous research (Gerdjikov and Beninger, 2005; Lin et al., 2003; Nakazawa et al., 1995). Pups were then placed with the dam until tested for odor preference, or were sacrificed for phosphorylated 3'-5'-cyclic AMP response element binding protein (pCREB) immunohistochemistry.

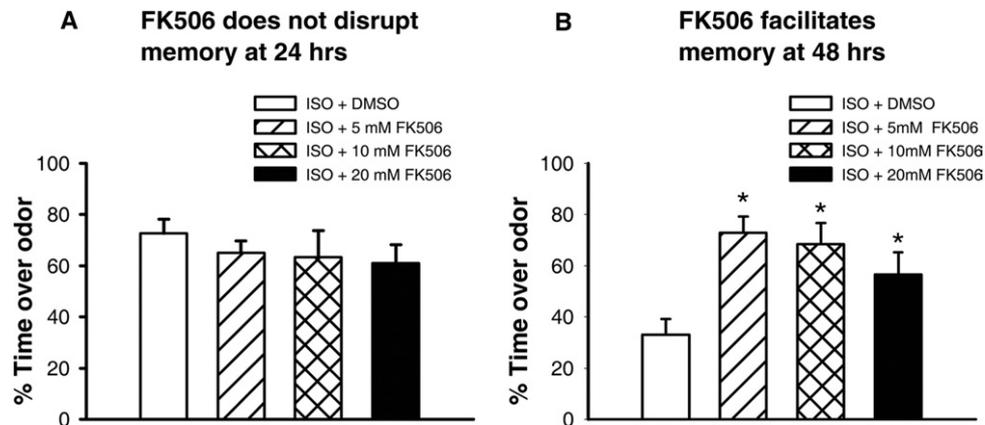
### Testing for conditioned odor preference

The procedure for odor preference testing was described previously (McLean et al., 2005). In brief, each pup underwent five 1-min trials of a two-odor choice test. Summation of the time spent over peppermint was divided by the total activity time (time spent out of a neutral zone) to give the percent time over peppermint (conditioned) odor. For long-term memory tests, each animal was tested for memory at one time point only while littermates were tested at other time points.

### Immunohistochemistry and relative optical density analysis of pCREB

Pups were given an infusion of vehicle into one bulb and FK506 into the other (the bulb receiving FK506 was randomly assigned among pups) immediately after odor preference training. In order to determine if 3'-5'-cyclic AMP response element binding protein (CREB) phosphorylation was extended more than the normal 10 min after training (McLean et al., 1999; Yuan et al., 2000), 40 min after infusion/odor exposure pups were removed from the dam and were killed by decapitation. Each brain was immediately removed from the skull and transferred into cold 4% paraformaldehyde in a 0.1 M phosphate buffer. Brains were left in fixative overnight and then were transferred into a 20% sucrose solution in 0.1 M phosphate buffer until they were cut 24 h later and processed for pCREB (Upstate) visualization as described previously (McLean et al., 1999).

Two middle (rostral to caudal) sections were selected per pup and slides were coded so the analyzer was blind to which bulb was infused with FK506. Image analysis (Bioquant, R&M Biometrics, Inc.) was performed by tracing the dorsomedial, ventromedial, dorsolateral and ventrolateral quadrants of the glomerular, mitral and granule cell layers. An area of the olfactory nerve layer was captured digitally and assigned as the background level for relative optical density measurements. The optical density of cells labeled with pCREB was captured digitally from each analyzed layer. The relative optical density of each region of interest was determined by subtracting the background level from the density of the pCREB labeled area of interest, and the difference was divided by the olfactory nerve background level (Yuan et al., 2003b).



**Fig. 1.** (A) Pups given 2 mg/kg ISO paired with odor and infused immediately after a 10 min training session with various concentrations of FK506 all showed preference for the associated odor 24 h after training, indicating that the CaN inhibitor did not interfere with memory ( $n=5$  pups/group). (B) The same training paradigm as in (A) showed that CaN inhibition extends memory to 48 h ( $n=9$  pups/group). Note that 10 min pairing of 2 mg/kg ISO and odor alone does not provide odor memory at 48 h (see vs. same training in A, 24 h memory).

### Statistical tests

One way or repeated measures analyses of variance (ANOVAs) were used to test for across group differences. Post hoc Dunnett's or Bonferroni tests probed for specific comparisons.

## RESULTS

### Surgery, intrabulbar infusion, and CaN inhibition do not interfere with the memory normally seen 24 h after training

Pups that received 2 mg/kg of ISO paired with peppermint and either vehicle (DMSO) or bilateral infusions of any of three concentrations (5, 10 or 20 mM) of FK506 on PND 6 showed a normal preference for the CS peppermint on PND 7. Thus, the pups showed preference for the CS that was not significantly different from the DMSO learning controls ( $F_{(3, 16)}=0.4846$ ;  $P=0.698$ ,  $n=5$  pups/group) indicating that infusion of CaN inhibitors did not interfere with preference acquisition or retention (Fig. 1A).

### Inhibition of CaN with FK506 extends the duration of odor preference memory

Pups that received 2 mg/kg ISO and 5, 10 or 20 mM of FK506 all showed significantly greater preference for the conditioned peppermint odor at 48 h than pups infused with 2 mg/kg ISO and vehicle ( $F_{(3, 31)}=4.212$ ;  $P<0.05$ ). These results also confirm previous findings (McLean et al., 2005) that pups given 2 mg/kg of ISO, when combined with odor, do not exhibit preference for peppermint 48 h after training. All three concentrations of CaN inhibitor facilitated extended memory (Fig. 1B).

### CaN inhibition, on its own, does not produce olfactory memory

To assess whether FK506 might act as a UCS on its own, pups were given either saline or 2 mg/kg ISO followed by odor exposure and either 5 mM FK506 or vehicle infusion. ANOVA ( $F_{(3, 52)}=6.223$ ;  $P<0.05$ ) and Bonferroni post hoc showed that only pups that received

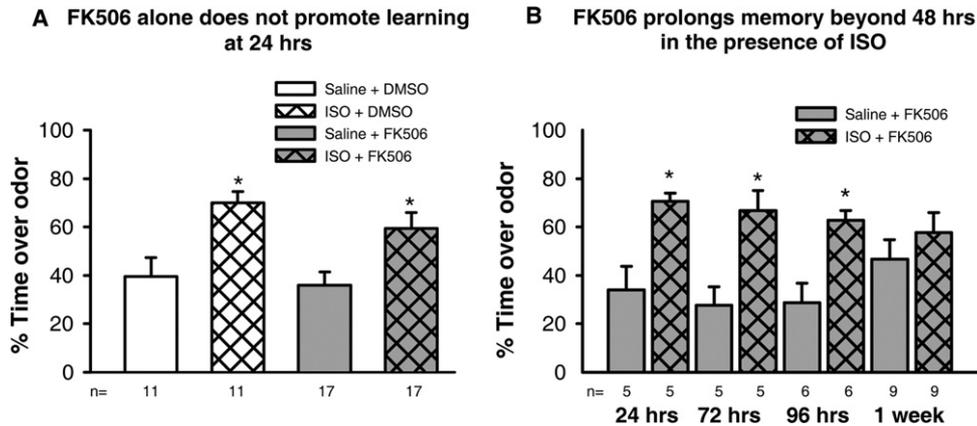
ISO as the UCS, with or without FK506, had odor preference memory 24 h after training (Fig. 2A).

### CaN inhibition with 5 mM FK506 retains memory beyond 48 h

We also investigated the effect of FK506 on longer memory retention. On PND 6, pups received an s.c. injection of saline or 2 mg/kg ISO, and an intra-bulbar bilateral  $1 \mu\text{l}$  infusion of 5 mM FK506. Pups were tested for odor preference 24 h, 72 h, 96 h, or 1 week after training (each pup was tested only at one time point). A one-way ANOVA ( $F_{(7, 42)}=4.237$ ;  $P<0.01$ ) with post hoc Bonferroni multiple comparison tests revealed a significant difference in preference for peppermint of pups infused with FK506 compared with pups infused with vehicle 24, 72 and 96 h after training (Fig. 2B). At 1 week after training, the effect of FK506 on memory was not observed, possibly because the non-learning control group showed a higher than usual preference for the peppermint odor at that time point.

### CaN inhibition prolongs the duration of CREB phosphorylation in the olfactory bulb following odor preference training

As shown in Fig. 3A, there were noticeable increases in the darkness of nuclei stained positively for pCREB in the granule, mitral and glomerular cell layers of bulbs infused with FK506 compared with those infused with vehicle. When measurements were combined across quadrants, repeated measures ANOVA of relative optical density levels showed significant differences between groups ( $n=4$  for each group;  $F_{(5, 12)}=4.443$ ;  $P<0.001$ ). Post hoc Bonferroni multiple comparison tests showed higher levels for bulbs infused with FK506 than vehicle in each of the three layers examined, mitral, granule and glomerular. The relative optical density of nuclei stained positively for pCREB was also significantly different overall in the glomerular layer ( $P<0.01$ ), mitral cell layer ( $P<0.05$ ) and the granule cell layer ( $P<0.01$ ). If considered by quadrants, a more selective pattern emerged.

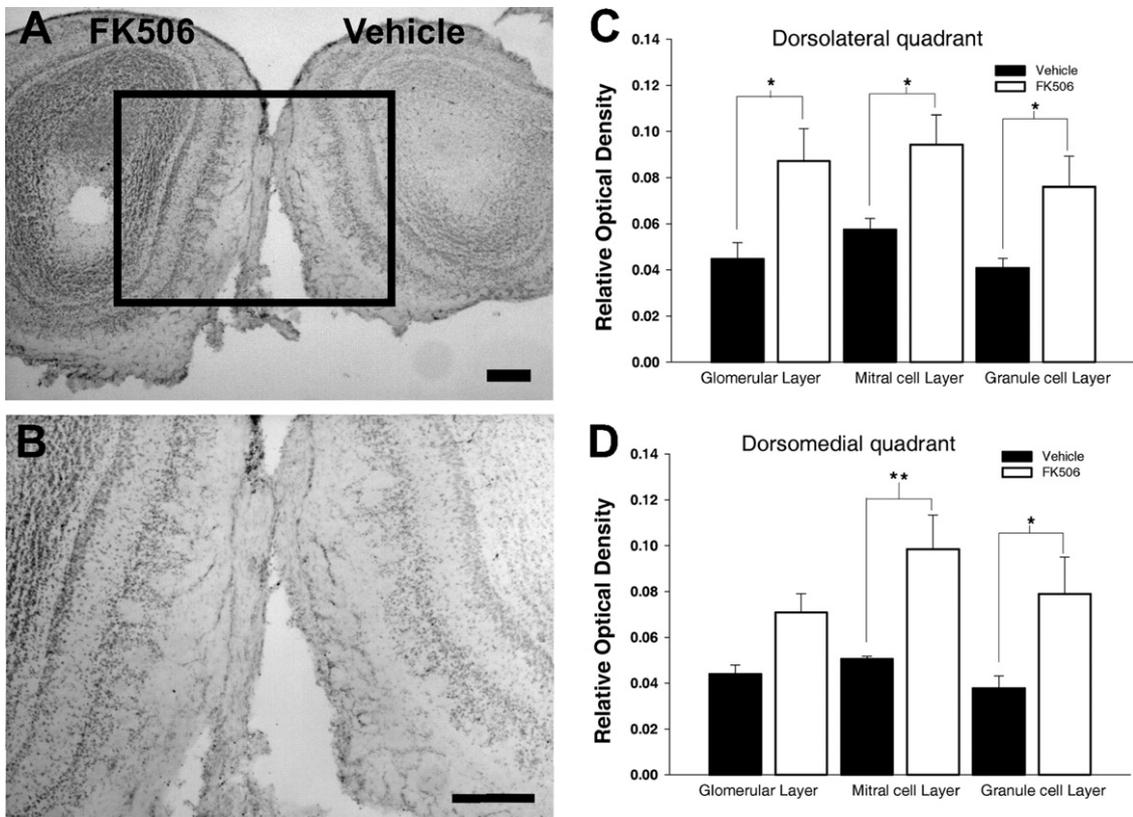


**Fig. 2.** (A) CaN inhibition, using 5 mM FK506 by itself, is not able to induce an odor preference memory 24 h following odor exposure. Pups given 2 mg/kg ISO did, however, show normal olfactory learning with either an infusion of FK506 (also observed in Fig. 1A) or vehicle. \*  $P < 0.05$ . (B) Preference for peppermint odor was examined 24, 72, 96 h and 1 week after training. All animals were infused with 5 mM FK506 immediately after training. Additionally, one group was given saline s.c. 40 min prior to training while the other group was given 2 mg/kg ISO. \*  $P < 0.05$ .

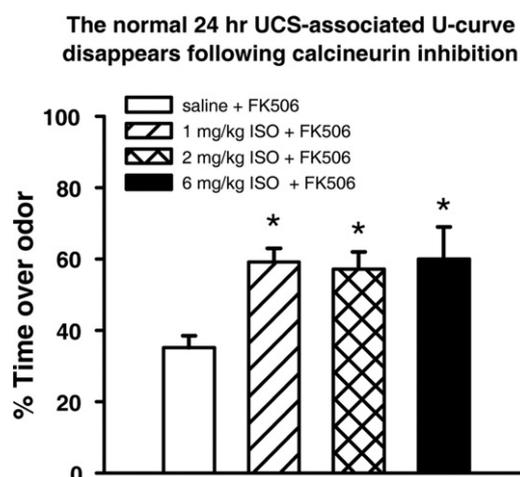
The relative optical density of nuclei stained for pCREB in the mitral and granule cell layers was only significantly higher with FK506 infusion in the dorsolateral and dorsomedial quadrants of the olfactory bulb (Fig. 3B). There were no significant differences between FK506 and vehicle in the ventromedial quadrant and only the glomerular layer showed higher pCREB levels in the ventrolateral quadrant.

**The UCS-associated normal inverted U-curve disappears with CaN inhibition**

Pups given s.c injection of 1, 2 or 6 mg/kg ISO and infused with 5 mM FK506 after odor exposure all showed significant preference for peppermint when tested 24 h after training when compared with control pups which received s.c. injection of saline and infusion of FK506 ( $F_{(3, 32)} = 4.212$ ;  $P < 0.05$ ).



**Fig. 3.** Forty minutes after training, pCREB expression is prolonged in the CaN-inhibited olfactory bulb. (A) Photomicrograph of pCREB expression in a representative section. Scale bar = 100  $\mu$ m (B) Higher magnification of inset of A. Scale bar = 100  $\mu$ m. Quantitative analysis of the relative optical density of pCREB expression in the dorsolateral (C) and dorsomedial (D) quadrants of the olfactory bulb. \*  $P < 0.05$ ; \*\*  $P < 0.01$ .



**Fig. 4.** CaN inhibition eliminates the normal UCS-induced inverted U-curve. ISO, as the UCS, is necessary for 24 h memory but the normal inverted U-curve is no longer observed when pups are trained with 5 mM FK506. Normally, 1 and 6 mg/kg ISO are ineffective as the UCS while 2 mg/kg ISO is optimal ( $N=9$  pups/group). \*  $P<0.05$ .

Post hoc analysis with the Dunnett's multiple comparison test revealed that pups given all three concentrations of ISO led to greater preference for peppermint when compared with non-ISO control pups (Fig. 4).

## DISCUSSION

These experiments had three clear outcomes. CaN inhibition strengthened the odor preference memory trace as reflected in approximately 400% increase in memory duration (4 days vs. 1 day). CaN inhibition prolonged the period of CREB phosphorylation in mitral and granule cells of dorsomedial and dorsolateral quadrants of the olfactory bulb. CaN inhibition eliminated the normal inverted U curve seen with 1 mg, 2 mg and 6 mg doses of the UCS  $\beta$ -adrenoceptor agonist, ISO. Previous work has repeatedly shown no early odor preference learning when odor is paired with 1 mg/kg ISO during training (McLean et al., 2005; Price et al., 1998; Sullivan et al., 1989; Yuan et al., 2003a) and no learning when odor is paired with 4 mg/kg ISO (Langdon et al., 1997; Sullivan et al., 1989; Yuan et al., 2003a) or 6 mg/kg ISO (Langdon et al., 1997; Yuan et al., 2000). Thus, in the present experiments, we now show learning occurs at a normally inadequate dose and at a normally excessive dose, while the optimal dose continues to be effective.

The increase in memory strength with CaN inhibition, as reflected in increased memory duration, parallels the recent effects of transgene CaN manipulations in mice using a one trial conditioned taste aversion paradigm (Baumgartel et al., 2008). Conditioned taste aversion training induces a very long lasting memory and a decrease in amygdalar CaN activity in membrane fractions following training in normal mice. A further decrease in CaN activity, prior to training, increases memory strength and resistance to extinction, an indirect index of memory strength. Conversely, increasing CaN activity decreases memory and

resistance to extinction. The extinction process itself is unaffected. The authors conclude that memory strength is related to the level of CaN activity, likely in specific memory-related structures like the amygdala. The mouse experiments did not look at transgene activation immediately posttraining. Other paradigms have also provided evidence of increases in memory strength, duration and ease of induction with CaN inhibition during acquisition (Gerdjikov and Beninger, 2005; Malleret et al., 2001; Sharma et al., 2003).

The present experiment provides evidence that CaN inhibition in the period immediately posttraining is sufficient to produce memory strengthening. An unexpected result was the induction of odor preference to a normally ineffective dose (1 mg/kg) of ISO UCS with a manipulation given after the training period. We suggest that the distinction between acquisition and consolidation may need to be reconsidered given these data. A framework that could account for both acquisition and memory extension effects seen here is related to parallel kinase/phosphatase activations. In the mitral cell model of odor preference learning (McLean and Harley, 2004), the critical cAMP signal has been shown to occur at the end of the 10 min training trial (Cui et al., 2007). This signal would alter the balance of kinase and phosphatase activation to favor kinase dominance. In the present study, reducing CaN phosphatase alters that balance in the normally threshold learning condition to create acquisition. Memory prolongation would be related to the dominance of the kinase signal. It would be of interest to ask whether memory duration is extended for 1 mg/kg ISO paired conditions. The present data demonstrate that inhibition of CaN in a specific memory-related structure, as postulated in the mouse studies, is sufficient to increase memory strength. Earlier work with pheromonal memory and FK506 infusion in the accessory olfactory bulb also supports the efficacy of structure-specific CaN inhibition on memory strength (Nakazawa et al., 1995), but intracerebral CaN inhibition appears deleterious for some memories e.g. working memory (Runyan and Dash, 2005).

With 24 h memory for one trial odor preference learning, CREB phosphorylation is maximal 10 min after the end of odor training with a return to basal pCREB levels by 60 min posttraining (McLean et al., 1999; Yuan et al., 2000). Significant increases of pCREB are found in mitral cells of the dorsolateral quadrant consistent with the localization of peppermint odor representation (McLean et al., 1999). There were also increases in other regions of the bulb, most likely due to mass infusion of CaN and distribution of ISO throughout the bulb. Here we find extension of normal memory duration by CaN inhibition is associated with a more enduring elevation of pCREB with significantly higher levels at 40 min posttraining. This result provides support for the hypothesis that the duration of memory or memory strength is determined by the duration of nuclear pCREB activation. Long-lived aversive memories following one-trial training are also associated with longer nuclear pCREB activation than seen with 24 h odor aversive memory (Zhang et al., 2003). The somewhat broader spatial

distribution of pCREB seen after CaN inhibition may relate to memory strength or may be a feature of pharmacological bulbar CaN inhibition.

CaN has been shown to regulate CREB dephosphorylation (Enslin et al., 1994; Bito et al., 1996). With CaN inhibition, memory was demonstrated up to 4 days after training, at 1 week memory differences were in the same direction, but highly variable. Other investigators have reported extension of 24 h memories to several days after CaN inhibition (Malleret et al., 2001), consistent with the possibility of 'intermediate' long-term memories. Alternatively, our behavioral test may not be sufficiently sensitive for pups at 1 week posttraining. At 4 days, but not 1 week, memory for odor preference was previously seen with PDE inhibition during training (McLean et al., 2005). These results relate the strength and duration of positive cAMP signaling to memory strength as indexed by duration. Whether the behavioral data support a spectrum of intermediate long term memory durations from 1 day to 4 days to lifelong memories that are associated with varying levels of dephosphorylation, or whether a lifelong memory is triggered with CaN inhibition, but not revealed in our testing, remains to be explored.

While CaN was first reported to act as a PDE inhibitor, its primary function is as a phosphatase (Rusnak and Mertz, 2000). The effects of immediate posttraining FK506 on odor preference acquisition are quite distinct from those of PDE inhibition. PDE inhibition promotes learning with a low 1 mg dose of the beta agonist ISO, but prevents learning at the normally effective 2 mg dose, consistent with a shift in the peak of the inverted U curve (McLean et al., 2005). CaN inhibition eliminates the inverted U curve, promoting the effectiveness of a low 1 mg dose, and of a high 6 mg dose, of ISO. Learning continues to be effective with a 2 mg dose of ISO, the only modulation being a strengthening of the memory at that dose. This is a remarkable result and argues that the dephosphorylations initiated by CaN determine both the threshold for odor preference learning and the memory suppressive effects of excessive activation of the cAMP system. Whether CaN is a general regulator of the ubiquitous behavioral inverted U curve seen with strong arousal remains to be tested. The promotion of weak memory signals by CaN has been seen previously (Sharma et al., 2003). The memory pattern seen here argues for a race between memory suppression and memory promotion events which is rapidly triggered posttraining in early odor preference learning.

What is the function of the race between memory promotion and memory suppression mechanisms? As argued previously such mechanisms are likely critical in shaping learning circuits based on Hebbian and anti-Hebbian plasticity (Lisman, 1989). It is also the case that memory suppression provides a way to regulate information storage as a function of event relevance or importance (Abel et al., 1998), contributing, as seen here, to the ineffectiveness of weak, but otherwise positive, UCS signaling via the cAMP cascade. The role of inverted U curve effects is less clear, but would attenuate memory in highly stressful circumstances which could mitigate against obsessive

and posttraumatic stress memory effects. Is there a decrease in the level of CaN activity with repeated spaced learning trials (Abel et al., 1998)? This is a testable hypothesis that would provide a novel mechanism for the effectiveness of spaced trials in promoting memory.

## CONCLUSION

In summary, assessment of the role of CaN, using direct inhibition of CaN activity via FK506 locally in olfactory bulb, reveals a role for CaN in regulating the inverted U curve of UCS effectiveness in acquisition and in regulating memory strength in a cAMP-mediated paradigm of learning and memory. A strong and significant role for this phosphatase in cAMP-mediated learning is consistent with its co-localization with protein kinase A in the postsynaptic density.

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