

**Long-term memories in dorsomedial and dorsolateral striatal areas
differentially affect behavioral flexibility**

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Long-term memories in dorsomedial and dorsolateral striatal areas differentially affect behavioral flexibility

Thesis co-directed by Dr. Randall C. O'Reilly and Dr. Jerry W. Rudy

Actions can be performed because they are expected to be rewarded, or simply because they have been expressed so many times that contextual cues elicit them automatically. Existing data suggest that the dorsomedial striatum (DMS) supports expectancy-driven behaviors, and that the dorsolateral striatum (DLS) allows contextual cues to elicit habitual behavior. However, it is still an open question whether or not the separable contributions of these neural substrates depend on a long-term memory of task-relevant information. Alternatively, this information may be stored in other areas that are interacting with these striatal regions. In order to address this question, rats were trained to press two levers associated with concurrent variable interval schedules of reinforcement. We first demonstrated that injecting the NMDA antagonist DL-AP5 in the DMS during testing renders rats insensitive to contingency changes, but when injected into the DLS, it enhanced their sensitivity to contingency changes, thereby implicating their differential in expectancy-driven and habitual responding. We then used the protein kinase zeta (PKM ζ) inhibitor zeta pseudosubstrate inhibitory peptide (ZIP) to determine if these regions provide storage support for the information needed to respond to contingency changes. Injecting ZIP into the DLS enhanced the rats' ability to adapt to the contingency shift, whereas injecting ZIP into the DMS had the opposite effect. It was possible to rescue the sensitivity to contingency changes by preventing the effect of ZIP with infusions of GluR2_{3Y} into the DMS one hour before ZIP microinfusions. This indicates that ZIP was preventing the ability of PKM ζ to maintain established memories in the dorsal striatum by regulating GluR2-dependent AMPA receptor trafficking. These results indicate that the two dorsal striatal areas actively maintain long-term memories that affect the sensitivity to contingency changes.

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This work is dedicated to the memory of my mother.

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Chapter 1

Introduction

A particular action can be performed because it is expected to be rewarded, or it can be performed habitually, independent of its consequences. Whether actions are expectancy-driven or habitual changes with repetition of the behavior. After subjects initially discover that a reward is contingent on an action, an action is chosen because it is expected to be rewarded. If multiple actions are available, they are chosen according to their probability of being rewarded. If relative reward rates for the different behavioral alternatives remain relatively constant in a stationary environment, stimulus-response associations develop gradually and elicit behavior directly. This habitual behavior is independent of a reward expectancy, but merely depends on the contiguity between behavior and reward in the behavioral context.

It is believed that expectancy-driven behavior is supported by the dorsomedial striatum (DMS) and habitual behavior by the dorsolateral striatum (DLS). Both regions are part of the dorsal striatum, which is the largest input nucleus of the basal ganglia, and controls input and output of information in frontal cortex (Hazy, Frank, & O'Reilly, 2007).

The DMS corresponds to the caudate in humans (Voorn, Vanderschuren, Groenewegen, Robbins, & Pennartz, 2004), and is thought to support expectancy-driven behaviors (Kawagoe, Takikawa, & Hikosaka, 1998; Kimchi & Laubach, 2009b; Lau & Glimcher, 2008; O'Doherty, Dayan, Schultz, Deichmann, Friston, & Dolan, 2004; Pauli, Hazy, & O'Reilly, submitted), because it participates in a network that includes frontal areas and amygdala (McGeorge & Faull, 1989; Alexander, DeLong, & Strick, 1986; Middleton & Strick, 2000; McDonald, 1991), areas that are involved in tracking performance, instrumental contingencies and reward expectancies (Balleine & Dickinson, 1998; Lapish, Durstewitz, Chandler, & Seamans, 2008;

Schoenbaum, Roesch, Stalnaker, & Takahashi, 2009).

The DLS is thought to support habitual responding that develops after extensive practice (Yin, Knowlton, & Balleine, 2004; Pauli, Hazy, & O'Reilly, 2009). The DLS is interconnected with sensory cortex and motor areas of frontal cortex (Alexander et al., 1986; Middleton & Strick, 2000), and corresponds to the putamen in humans (Voorn et al., 2004).

1.1 The Experimental Question

Even though ample evidence suggests that these two dorsal striatal regions make separable contributions to behavior, it is still an open question whether either striatal region is a long-term store of information that affects behavioral flexibility (Shiflett & Balleine, 2011). One view is that the striatum may only be necessary for rapidly identifying rewarded associations initially, but then enable and train slower learning frontal cortical areas (Pasupathy & Miller, 2005). A second view is that the DMS has to maintain how to interpret task-related activation in medial frontal areas and the basolateral amygdala (BLA) in order to support expectancy-driven behavior, and that the DLS maintains stimulus-response associations in order to support habitual responding (Pauli et al., 2009; Pauli, Atallah, & O'Reilly, 2010; Pauli et al., submitted).

1.2 The Strategy - PKM ζ

The goal of the experiments reported here was to answer this question. What is needed to approach this question is a methodology that in principle can erase memories for task-relevant information without damaging the neurons, and allow the animal to function normally otherwise. Ablating or temporarily inactivating these dorsal striatal areas are therefore not apt for approaching this problem. However, recently there is emerging evidence that inhibiting the memory maintenance properties of a unique kinase called PKM ζ provides an ideal approach to this problem (Pastalkova, Serrano, Pinkhasova, Wallace, Fenton, & Sacktor, 2006; Serrano, Friedman, Kenney, Taubenfeld, Zimmerman, Hanna, Alberini, Kelley, Maren, Rudy, Yin, Sacktor, & Fenton, 2008; Hardt, Migues, Hastings, Wong, & Nader, 2010; Shema, Sacktor, & Dudai, 2007; Li, Xue, He, Li, Xue, Xu, Sacktor, Shaham, & Lu, 2011; Shema, Haramati, Ron, Hazvi, Chen, Sacktor, &

Dudai, 2011).

Currently PKM ζ is the only known protein that addresses the molecular turnover problem of long-term memory (Crick, 1984): How can the information encoded as changes in synaptic strength be maintained when all the components of the synapse are subject to inevitable molecular turnover? Even though many proteins have been discovered that are critical for the induction of LTP (Kelleher, Govindarajan, Jung, Kang, & Tonegawa, 2004; Nguyen & Woo, 2003; Patterson, Grover, Schwartzkroin, & Bothwell, 1992; Tully, 1991), inhibiting any of these proteins once memories have been established does not lead to a retrograde memory impairment (Sacktor, 2011). However, the inhibition of PKM ζ has been shown to eliminate established LTP and erase memories (Sacktor, 2011).

Considerable progress has been made in understanding the specific role of PKM ζ in the active maintenance of memories. The potentiation of a synapse leads to the activation of homeostatic processes that increase the endocytosis of AMPA receptors (2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl)propanoic acid) that would be critical for maintaining an increased fast synaptic transmission (Migues, Hardt, Wu, Gamache, Sacktor, Wang, & Nader, 2010). PKM ζ maintains the synaptic potentiation despite this increased endocytosis by regulating GluR2-dependent trafficking of AMPA receptors into the synapse (Migues et al., 2010). Inhibiting this process by infusing the PKM ζ inhibitor, zeta pseudosubstrate inhibitory peptide (ZIP), leads to a LTD-related memory loss. Simultaneously inhibiting the endocytosis of GluR2-containing AMPA receptors with GluR2_{3Y} while also inhibiting PKM ζ prevents LTD-related memory loss (Li et al., 2011; Migues et al., 2010). Because both ZIP and GluR2_{3Y} only affect maintenance of potentiated synapses and do not lead to impairments in untrained animals (Brebner, Wong, Liu, Liu, Campsall, Gray, Phelps, Phillips, & Wang, 2005; Yu, Wu, Liu, Ge, & Wang, 2008; Migues et al., 2010), they represent an excellent analytic tool for investigating the contents of dorsal striatal long-term memories without affecting their overall function. Furthermore, ZIP can be infused several days before the contingency change, so that the drug is washed out at the time of behavioral testing. Because of the selectivity of ZIP and the lack of an acute drug effect, changes in the ability to adapt to new contingencies are unlikely to be caused by a general striatal dysfunction.

1.3 The Behavioral Methodology

To investigate whether either striatal region stores long-term associations, we developed a paradigm that allows one to assess the ability of animals to adjust to changes in the contingencies associated with its behavior. Specifically, we trained rats to press two levers associated with concurrent variable interval schedules of reward. After several consecutive sessions with unchanged relative reward rates, expectancy-driven and habitual responding should work in synergy. The expectancy system supports an action because it is expected to be rewarded, and the habit system elicits behavior directly according to how often it has been rewarded in the current context. We then tested the ability of rats to adapt to an eventual contingency change. The expectancy system should notice the violation of its expectancies in the recent reward history, update its expectancies, and thus promote a rapid adaptation of behavior. At the same time the habit system should continue to elicit behaviors according to the previous contingencies, and retard an adaptation. Thus, when contingencies change the synergy between the two systems should devolve into competition, and behavior should be the result of a combined influence of the two systems.

In the first experiment we tested whether the two striatal areas differentially contribute to an adaptation to a contingency shift. For this purpose we microinfused the NMDA (N-Methyl-D-aspartic acid) receptor antagonist DL-AP5 (DL-2-Amino-5-phosphonopentanoic acid) into either striatal region 5 minutes before the contingency shift session. If the DMS supports a rapid adaptation to a contingency shift, NMDA antagonism in this region should render rats insensitive to contingency changes, behavior should be dominated by the DLS-supported habit system (Palencia & Ragozzino, 2005; Yin, Knowlton, & Balleine, 2005a; Yin, Ostlund, Knowlton, & Balleine, 2005b). If the DLS supports habitual responding, NMDA antagonism in the DLS should increase the rats' sensitivity to contingency changes, behavior should be less habitual (Palencia & Ragozzino, 2004; Yin et al., 2004).

DL-AP5 has been used in previous studies that investigated the contributions of these striatal regions to reversal learning (Palencia & Ragozzino, 2004, 2005). It has been found to not only block the induction of LTP, but also the expression of learned behaviors (Matus-Amat, Higgins, Sprunger, Wright-Hardesty, & Rudy, 2007) and synaptic transmission in medium spiny neurons (MSNs), the primary cell-type in the striatum.

tum (Tseng, Snyder-Keller, & O'Donnell, 2007; Kim & Jung, 2006; Chapman & Bellavance, 1992). These neurons alternate between a hyperpolarized resting membrane potential ("down state") (Kreitzer, 2009; Nisenbaum & Wilson, 1995) and transient periods of a sustained depolarization ("up state"; Surmeier, Eberwine, Wilson, Cao, Stefani, & Kitai, 1992; Wickens & Wilson, 1998; Carr, Day, Cantrell, Held, Scheuer, Catterall, & Surmeier, 2003), during which MSNs show an increased responsiveness to synaptic input. Antagonism of D1 dopamine and AMPA glutamatergic receptors have been found to block transitions into up states (Surmeier, Ding, Day, Wang, & Shen, 2007; Tseng et al., 2007), and NMDA receptor antagonism with DL-AP5 has been reported to significantly shorten the duration of up states (Tseng et al., 2007). Overall, the available data on DL-AP5 suggest that it reduces the contribution of a target region to behavior, and the amount of plasticity.

In the second experiment we tested whether the two striatal areas actively maintain a memory of task-related information that affects the ability of rats to adapt to a contingency change. We microinfused either the PKM ζ inhibitor ZIP or an inactive scrambled version of the peptide (scr-ZIP) into the DMS or DLS one day after the last training session. Two days later, when the drugs were washed out, we tested the effect of PKM ζ inhibition in either striatal region on the sensitivity towards a contingency change. If the DMS is a long-term store for information that is critical to support a rapid adaptation to contingency changes, ZIP infusions into this area should render rats less sensitive to contingency changes. If PKM ζ actively maintains a long-term memory in the DLS that supports habitual responding, ZIP infusions into this region should allow rats to adapt more quickly to the contingency change.

In the third experiment we tested whether PKM ζ maintains memories in the dorsal striatum by regulating GluR2-dependent AMPA receptor trafficking (Migues et al., 2010; Li et al., 2011). If ZIP infusions into the DMS blocked the ability of PKM ζ to regulate GluR2-dependent AMPA receptor trafficking, microinfusions of GluR2_{3Y} into the DMS one hour before ZIP should rescue the rats' ability to adapt to a contingency shift, because GluR2_{3Y} prevented the endocytosis of AMPA receptors and thus eliminate the dependency of memory maintenance on GluR2-dependent receptor trafficking by PKM ζ .

Chapter 2

Results

2.1 DMS and DLS differentially affect sensitivity to instrumental contingencies

To confirm that the DMS and DLS differentially contribute to the adaptation to contingency changes in our paradigm, we trained rats to press two levers on consecutive variable interval schedules of 20 seconds (VI 20"). Each time a rat was rewarded for pressing a lever, this lever would be inactive for an average of 20 seconds, but no more than 40 seconds. Pressing one lever, or being rewarded on one lever did not influence the probability of reward at the other lever. The next interval started as soon as a lever-press produced a reward. After training rats on this schedule for several sessions and a stable level of performance was established, the critical phase of the experiment began: we forced rats to adapt to a new set of contingencies. In particular, we changed contingencies so that the lever a rat had preferred during acquisition was now associated with a less favorable VI 40" schedule, and the other with a richer VI 10" schedule.

To determine whether the DMS and the DLS are differentially involved in adapting to the new contingencies, we either infused the NMDA antagonist DL-AP5 or saline into the DMS or DLS five minutes before this session, and then measured their ability to adapt to this contingency change, by pressing the richer lever VI10" more frequently than the other lever.

If the DMS supports a rapid adaptation to a contingency shift, microinfusions of DL-AP5 into this region before a contingency shift should impair a rat's ability to adapt to this shift. In contrast, if the DLS supports habitual behavior, microinfusions of DL-AP5 into this area should eliminate its contribution and enable rats to adapt more readily to a contingency shift.

In the last session before contingencies were changed, there was no difference among groups in their overall number of lever-presses per minute ($M = 3.48$, $SE = 0.23$; $F(3,16) = 1.82$, $p = .18$), and they responded on the two levers with about the same number of lever presses per minute ($t = -0.78$, $df = 19$, $p\text{-value} = 0.45$).

As shown in Figure 2.1, the predicted results were obtained. When DL-AP5 was infused into the DLS, rats adapted more readily to the contingency shift than controls, but when infused into the DMS it impaired the rats' ability to adapt to the contingency shift. Statistical support for this conclusion was provided by a linear mixed-effects model. Rats that received DL-AP5 infusions in the DLS shifted from pressing both levers about equally often on the last day before the contingency shift to pressing the more favorable VI 10" lever with a higher probability than the other lever after the shift (lme (day fixed effect): $t = 3.89$, $df = 4$, $p = 0.02$). In contrast, control rats that received saline vehicle infusions into the DLS did not show a significant adaptation to the new contingencies (lme (day fixed effect): $t = 1.23$, $df = 4$, $p = 0.29$). That is, rats that received DL-AP5 infusions adapted better to the contingency shift than control rats (lme (day * group fixed effect): $t = -2.44$, $df = 8$, $p = 0.04$). Rats that received DL-AP5 infusions into the DMS adapted worse to the contingency shift than rats that received control saline infusions (lme (day * group fixed effect): $t = 2.47$, $df = 8$, $p = 0.04$). While control rats that received saline infusions into the DMS adapted to the contingency shift (lme (day fixed effect): $t = 3.32$, $df = 4$, $p = 0.03$), rats that received DL-AP5 infusions into this area did not show a significant adaptation to the contingency shift (lme (day fixed effect): $t = -0.99$, $df = 4$, $p = 0.37$).

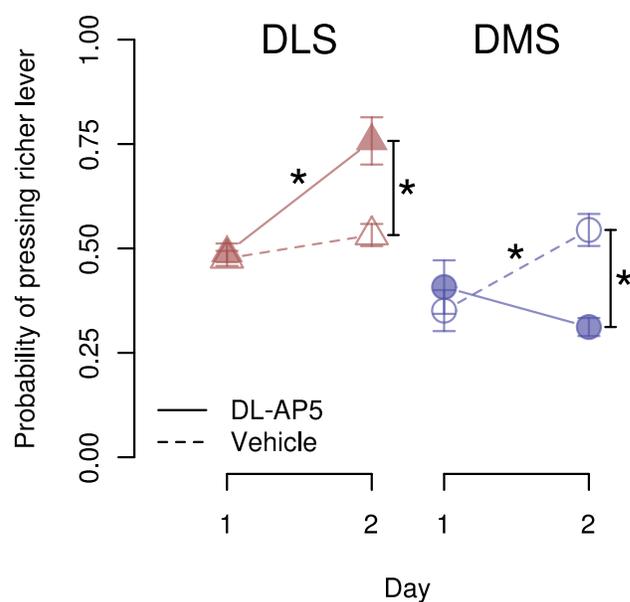


Figure 2.1: Probability of pressing the richer VI 10" lever. On the day before the contingency shift, when both levers were associated with a concurrent VI 20" schedule of reinforcement, rats in all groups pressed the lever that was going to be associated with the richer VI 10" schedule on the next day with about the same probability as the lever that was going to be associated with the less favorable VI 40". When contingencies were changed on the second day, rats that received DL-AP5 infusions into the DLS showed the greatest shift to pressing the more favorable lever with a higher probability than the less favorable lever. If DL-AP5 was infused into the DMS, rats did not adapt to the contingency shift. After saline vehicle infusions into the DMS or DLS, rats demonstrated a moderate adaption to the contingency change. Error bars indicate standard error (SE); (*) $p < 0.05$.

Figure 2.2 provides some insight into how rats that received DL-AP5 infusions into the DLS adapted differently to the contingency shift than rats that received DL-AP5 infusions into the DMS. Rats in these two groups did not differ in the number of times they pressed the lever associated with the less favorable VI 40" schedule, or the number of rewards they received from it. However, rats that received DLS infusions of DL-AP5 pressed the lever associated with the more favorable VI 10" more often than the VI 40" lever. Rats that received DL-AP5 infusions into the DMS did not show this increased probability of pressing the more favorable VI 10", despite the high ratio of rewarded to non-rewarded lever-presses.

As shown in Figure 2.3, DL-AP5 infusions into either area reduced the number of lever-presses per minute. This illustrates a common problem associated with testing animals under the influence of a drug. Nevertheless it seems unlikely that the reduced responding itself can account for the selectivity of the drug effect. This is because DL-AP5 enhanced the adaptation when infused into the DLS, but impaired the adaptation when infused into the DMS.

In summary, DL-AP5 infusions in the DMS reduced the rats' ability to adapt to the contingency shift. In contrast, DL-AP5 infusions in the DLS enhanced the ability of rats to adapt to the contingency change. Thus, our findings support the current view that these two regions of the striatum make different contribution to the processes that support behavioral adaptations.

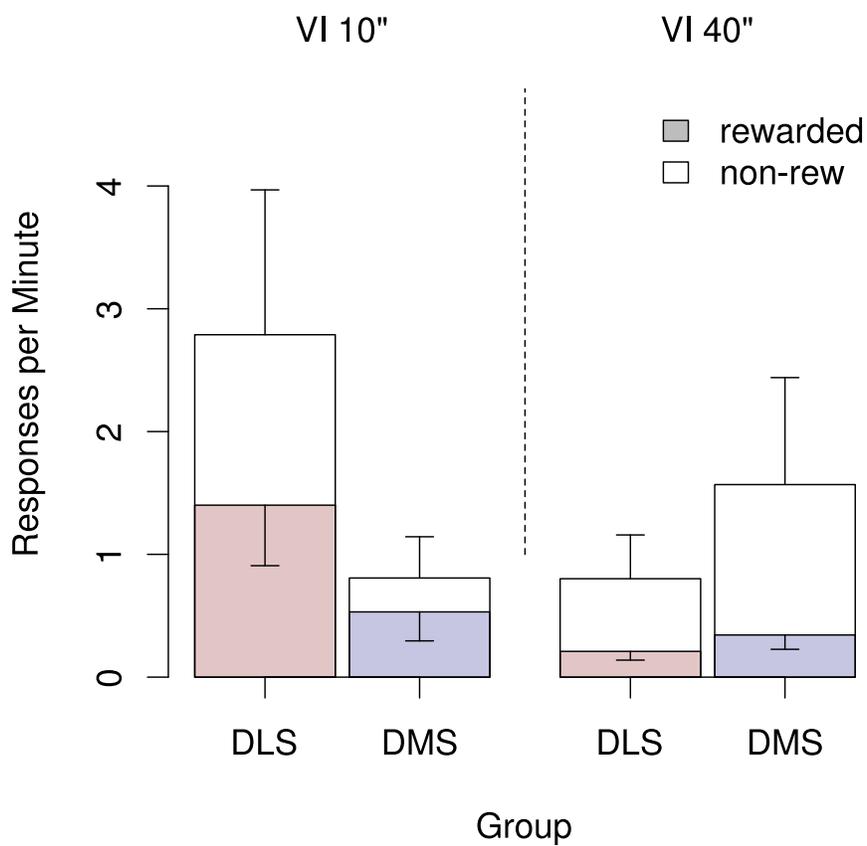


Figure 2.2: Rewarded and non-rewarded responses per minute. Microinfusions of DL-AP5 into either striatal region did not affect the number of lever-presses of the lever associated with the less favorable VI 40" schedule of reinforcement. In contrast, only rats that received DL-AP5 microinfusions into the DLS showed an increase in the number of responses on the VI 10" lever. Error-bars indicate standard error.

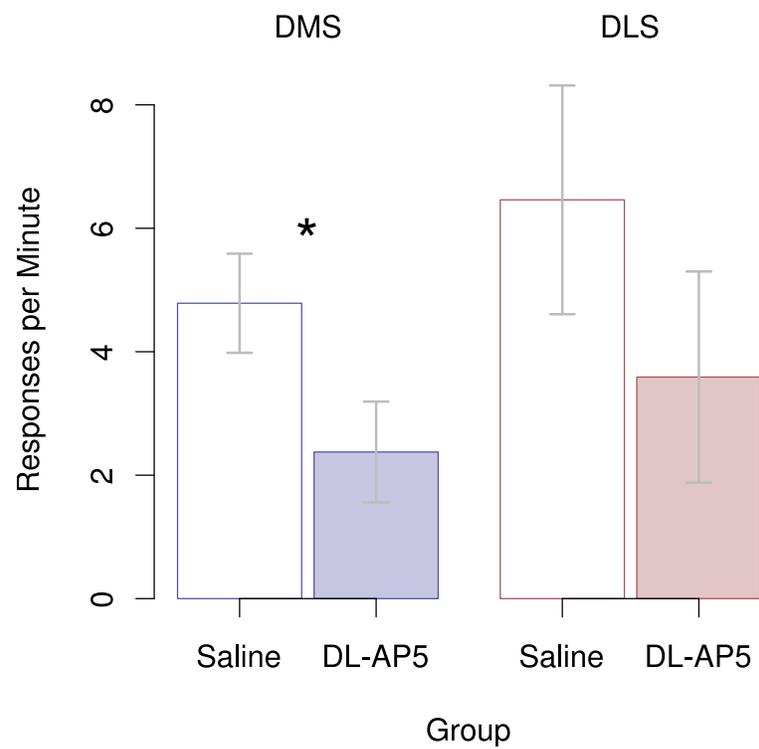


Figure 2.3: Mean number of responses per minute during the contingency shift session. Relative to saline vehicle injections, DL-AP5 lead to a reduction in the mean number of lever-presses per minute in the DMS group. (*) p-value < 0.05.

2.2 DLS and DMS maintain long-term memories of task-relevant information

The results of the previous experiment are consistent with the established view that the DMS and DLS make different contributions to behavioral adaptations to contingency changes. These data, however, do not address the question of main interest: Do these two dorsal striatal areas store information relevant to performance?

To address this question, we inhibited the memory maintenance protein PKM ζ . As noted above, other researchers have used this approach to erase memories for a variety of tasks and brain regions. Thus we reasoned that if the DMS and DLS actually stored task-relevant information, inhibiting PKM ζ should produce predictably different results. Specifically, inhibition of PKM ζ in the DLS should allow rats to adapt more readily to new contingencies, but inhibition of PKM ζ in the DMS should slow down an adaptation. To pursue this strategy we made a subtle change in the training strategy to increase the challenge imposed by the contingency shift. We trained rats on concurrent interval schedules of reinforcement, and after they had learned to press both levers for reward, they were trained for several consecutive sessions during which one lever was associated with a richer VI 10" schedule and the other lever with a VI 40" schedule of reinforcement. During this bias training, the lever a rat had preferred during the initial acquisition was associated with the less favorable VI 40", and the other with the VI 10" schedule. One day after the last day of bias training we either injected ZIP or an inactive scrambled version of the peptide (scr-ZIP) into the DMS or the DLS. In the next session 2 days later, contingencies were changed: the lever that had previously been associated with the more favorable VI 10" schedule was now associated with a VI 40" schedule, and the lever that had previously been associated with the less favorable VI 40" was now associated with a VI 10". If the DMS stores information long-term that is necessary for it to support a rapid adaptation to changing contingencies, microinfusions of ZIP into this region should impair the rat's ability to adapt to a contingency shift. If the DLS stores information long-term that makes behavior less sensitive to contingency changes, reversing LTP with ZIP injections into this region should make rats more sensitive to contingency changes.

As shown in Figure 2.4, rats rapidly adapted to the contingencies of the bias training by pressing the lever associated with the more favorable VI 10" schedule more than the other lever. Rats maintained this

preference through the course of the bias training. Figure 2.5 shows that at the end of bias training, rats had pressed the better lever significantly more often (Paired t-test: $t = 6.34$, $df = 18$, $p = 0.01$), and had also received significantly more rewards from it than from the other lever (Paired t-test: $t = -3.22$, $df = 18$, $p = 0.01$).

As shown in Figure 2.6, the predicted results were obtained. Rats adapted to the contingency shift if they received DLS infusions of ZIP (lme (day fixed effect): $t = 12.86$, $df = 5$, $p = 0.01$) or scr-ZIP (lme (day fixed effect): $t = 12.13$, $df = 3$, $p = 0.01$). However, rats that received ZIP infusions in the DLS adapted more strongly to the contingency change than rats with scr-ZIP microinfusions (lme (group * day interaction): $t = 3.16$, $df = 8$, $p = 0.01$). Rats that received microinfusions of ZIP into the DMS also adapted to the contingency shift (lme (day fixed effect): $t = 6.16$, $df = 6$, $p = 0.01$), as did rats that received DMS scr-ZIP infusions (lme (day fixed effect): $t = 11.48$, $df = 3$, $p = 0.01$). However, rats that received scr-ZIP infusions in the DMS adapted better than rats that received ZIP infusions (lme (group * day interaction): $t = -2.51$, $df = 8$, $p = 0.04$).

Figure 2.7 shows that the group that received microinfusions of ZIP into the DLS adapted to the contingency change early in the session and maintained this adaptation throughout the duration of the session. In contrast, rats that received ZIP infusions in the DMS shifted to pressing both levers about equally often, instead of pressing the more favorable VI 10" lever more than the other lever.

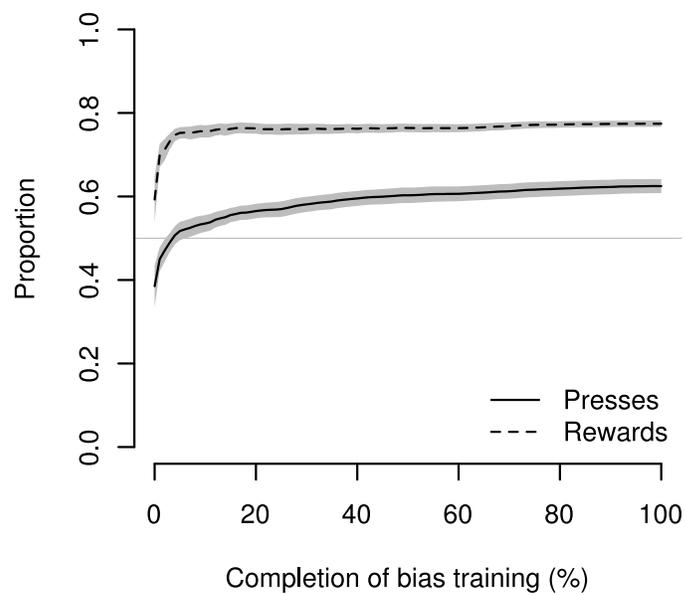


Figure 2.4: Soon after the beginning of bias training rats shifted to press the more favorable VI 10 lever at a higher proportion than the other lever. The proportion of reinforcements received from this lever increased accordingly. Y-axis shows the cumulative proportion of presses of the more favorable VI 10" lever, and proportion of reinforcements received from it. Shaded area indicates standard error (SE).

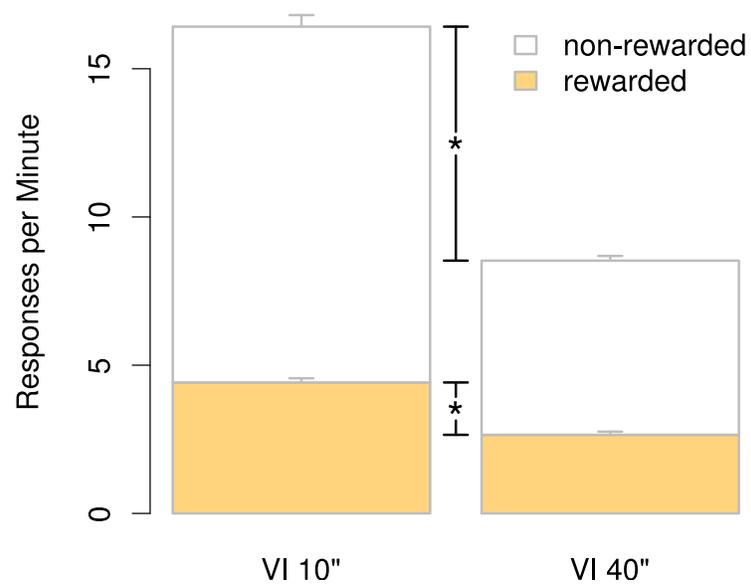


Figure 2.5: Rewarded and non-rewarded responses per minute during bias training. Rats pressed the more favorable VI 10" lever significantly more often than the other lever, and also received significantly more rewards from it. Error bars indicate standard error (SE); (*) $p < 0.05$.

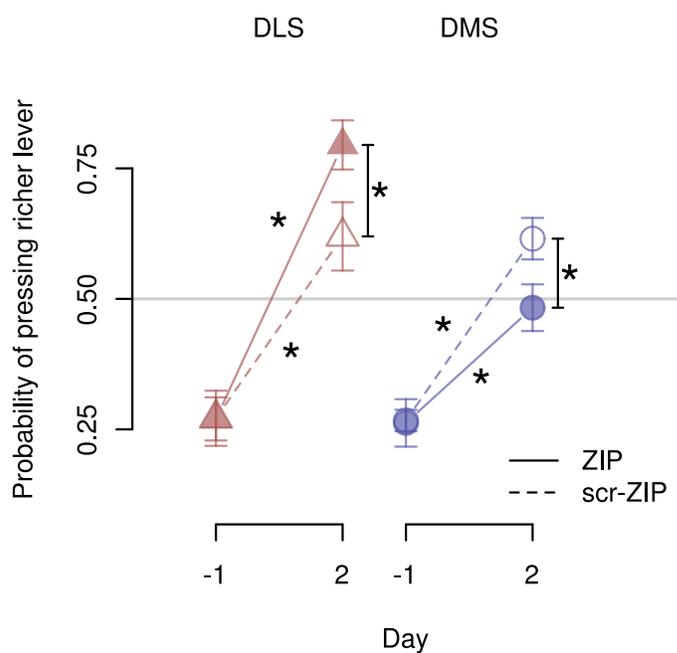


Figure 2.6: Probability of pressing the lever that was associated with the VI 10'' during the reversal session. On the day before the reversal session, when this lever was associated with the VI 40'', rats in all groups showed a similar low probability of pressing this lever. If ZIP was microinfused into the DLS, rats showed an enhanced ability to adapt to the contingency change, relative to control scr-ZIP injections. If ZIP was microinfused into the DMS rats showed a decreased ability to adapt to the shift. Error bars indicate standard error (SE).

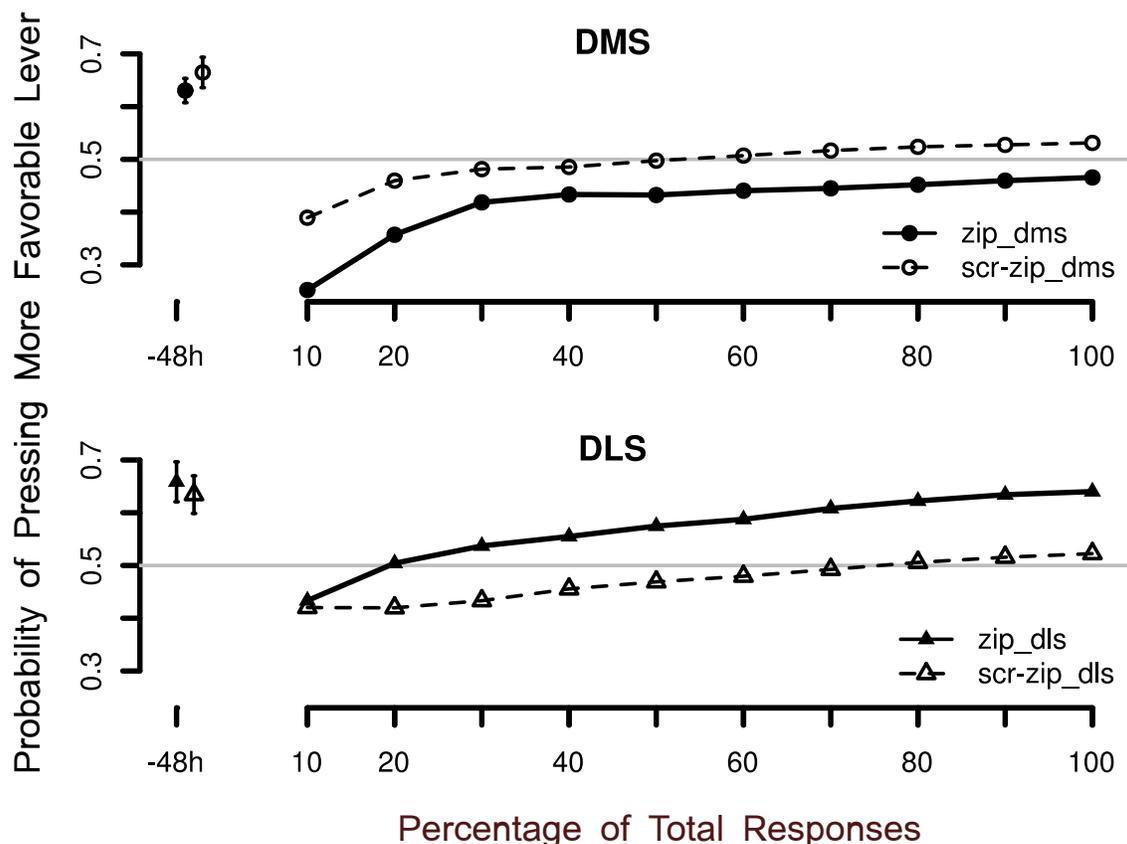


Figure 2.7: The amount of adaptation to the contingency shift changes with session progress. Rats that received ZIP infusions in the DLS adapted quickly to the contingency shift by pressing the more favorable lever more frequently than the other lever, and maintained this adaptation throughout the course of the session. In comparison, rats that received ZIP infusions in the DMS only show a slow increase over time in the probability of pressing the more favorable lever more frequently than the other lever. A direct comparison of the two groups at different levels of progress through the contingency shift session indicates that the difference between the two groups appeared early in the session and persisted until the completion of the contingency shift session. Shaded area indicate standard error (SE); (*) p-value < .05, (+) p-value < .1.

Figure 2.8 provides some insight into how rats that received DL-AP5 infusions into the DLS adapted differently to the contingency shift than rats that received DL-AP5 infusions into the DMS. The inability of the rats to adapt to the new contingencies after microinfusions of ZIP into the DMS appears to be the result of rats pressing both levers at an equally high rate (Paired t-test: $t = 0.79$, $df = 5$, $p = 0.47$), despite the difference in relative reinforcement rates (Paired t-test: $t = 3.35$, $df = 5$, $p = 0.02$). In contrast, the rats that received ZIP infusion in the DLS pressed the more favorable VI 10" more often than the VI 40" (Paired t-test: $t = 5.54$, $df = 5$, $p = 0.01$).

As displayed in Figure 2.9 the average number of lever-presses per minute after ZIP infusions was not significantly lower after ZIP infusions than after scr-ZIP microinfusions in the DLS (Welch Two Sample t-test: $t = -0.96$, $df = 5.4$, $p = 0.38$) or DMS (Welch Two Sample t-test: $t = -1.96$, $df = 7.15$, $p = 0.09$). There was also no difference in how often rats pressed levers depending on whether they received ZIP infusions in the DLS and rats that received ZIP infusions in the DMS.

In summary, inhibition of PKM ζ in the DMS impaired the rats' ability to adapt to the new contingencies. In contrast, inhibition of PKM ζ in the DLS enhanced the rats ability to adapt to the shift. These results suggests that PKM ζ is involved in actively maintaining a long-term memory for task-relevant information in the two dorsal striatal areas that underlies their differential contribution to behavioral flexibility.

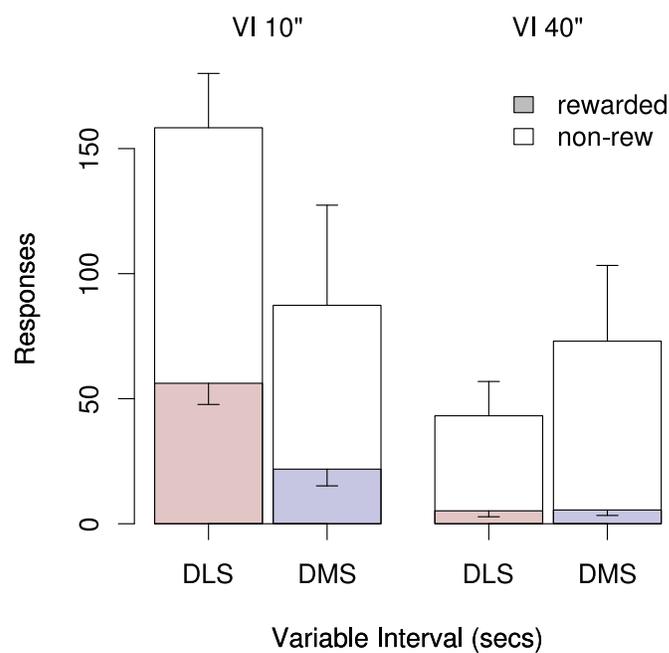


Figure 2.8: Rewarded and non-rewarded responses per minute at either lever. Rats with microinfusions of ZIP into the DMS pressed both levers similarly often, despite the difference in ratio of rewarded to non-rewarded responses. Rats with microinfusions of ZIP into the DLS increased responding on the lever with the VI 10'', and reduced responding on the lever associated with the VI 40'' schedule.

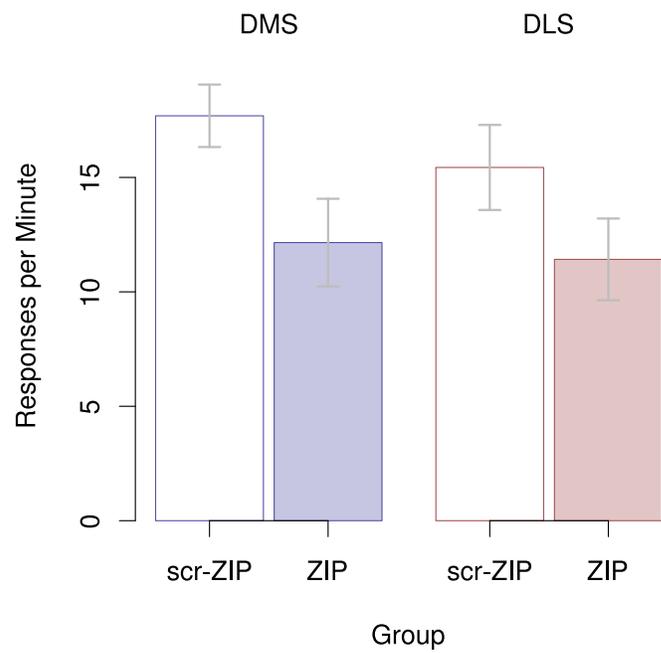


Figure 2.9: Mean number of responses per minute in the contingency shift session. Relative to scr-ZIP injections, injections of ZIP did not lead to a significant reduction in the average number of lever-presses per minute.

2.3 Simultaneous microinfusions of GluR2_{3Y} and ZIP into DMS abolish the reversal deficit found after ZIP injections

The previous results suggest that PKM ζ actively maintains a long-term memory of task-relevant information in the DMS and the DLS that allows these areas to differentially contribute to the adaptation to new contingencies. However, was the long-term memory in the two areas erased by interfering with the ability of PKM ζ to regulate GluR2-dependent AMPA receptor trafficking, or through some less specific process?

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To address this question, we blocked GluR2-dependent AMPA receptor endocytosis with GluR2_{3Y} while simultaneously inhibiting PKM ζ . As noted above, this procedure has been applied by other researchers to determine whether the ZIP-induced memory loss is caused by blocking the ability of PKM ζ to regulate GluR2-dependent AMPA receptor trafficking, and an associated reduction of post-synaptic GluR2 AMPA receptors. If PKM ζ maintains long-term memories in the DMS by regulating GluR2-dependent AMPA receptor trafficking, simultaneously blocking the removal of GluR2 AMPA receptors from the synapse with infusions of GluR2_{3y} while inhibiting PKM ζ should abolish the reversal deficit found when only PKM ζ was inhibited.

As in the previous experiment, rats were trained for several consecutive sessions during which one lever was associated with a VI 10" and the other lever with a VI 40". One day after the last day with these schedules and 2 days before the contingency shift session, we either microinfused saline or GluR2_{3Y} into the DMS one hour before ZIP microinfusions. Contingencies were reversed as in the previous experiment: the lever that had previously been associated with the more favorable VI 10" schedule was now associated with a VI 40" schedule, and the lever that had previously been associated with the less favorable VI 40" was now associated with a VI 10" schedule.

Rats rapidly adapted to the contingencies of the bias training by pressing the lever associated with the more favorable VI 10" schedule more than the other lever. Rats maintained this preference through the

course of the bias training (Figure 2.10). Figure 2.11 shows that at the end of bias training, rats had pressed the better lever significantly more often (Paired t-test: $t = 6.22$, $df = 12$, $p = 0.01$), and had also received significantly more rewards from it than from the other lever (Paired t-test: $t = 13.53$ $df = 12$, $p = 0.01$).

Figure 2.12 shows that the predicted results were obtained. Rats adapted to the new contingencies if they received GluR2_{3Y} infusions in the DMS (lme (group fixed effect): $t = 9.13$, $df = 6$, $p = 0.01$) or saline infusions (lme (group fixed effect): $t = 6.88$, $df = 5$, $p = 0.01$) one hour before ZIP infusions. However, rats that received GluR2_{3Y} microinfusions into the DMS adapted more strongly to the contingency change than rats that received saline microinfusions instead of the GluR2_{3Y} microinfusions (linear mixed-effects model (group * day interaction): $t = -2.48$, $df = 11$, $p = 0.03$).

Figure 2.13 indicates that the inability of the rats with microinfusions of ZIP into the DMS appears to be the result of rats pressing both levers at an equally high rate (Paired t-test: $t = 0.29$, $df = 5$, $p = 0.78$), despite the difference in relative reinforcement rates (Paired t-test: $t = 14.18$, $df = 5$, $p = 0.01$). Rats that received GluR2_{3Y} infusions before ZIP infusions, on the other hand, pressed the more richer VI 10" lever more than the other lever (Paired t-test: $t = 4.67$, $df = 6$, $p = 0.01$).

In summary, infusing GluR2_{3Y} into the DMS one hour before ZIP prevented the ZIP-induced memory-loss, and rescued the rats' ability to adapt to the contingency change. These data are consistent with the hypothesis that ZIP infusions inactivated PKM ζ and prevented it from regulating GluR2-dependent AMPA receptor trafficking in order to actively maintain a long-term memory for task-relevant information in the two dorsal striatal areas that underlies their differential contribution to behavioral flexibility.

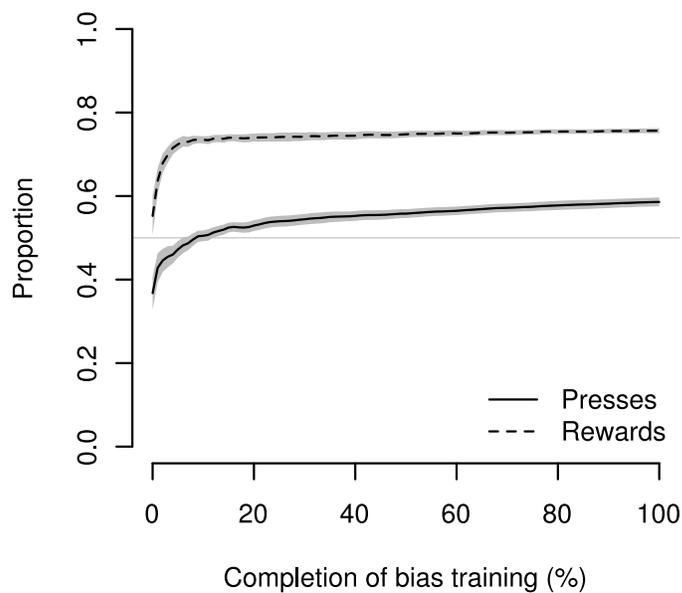


Figure 2.10: Proportion of presses of the lever associated with the VI 10" schedule and proportion of rewards received from that lever. Rats rapidly adapted to the contingency shift at the beginning of bias training, and maintained their preference over the course of bias training. Shaded area indicates standard error (SE).

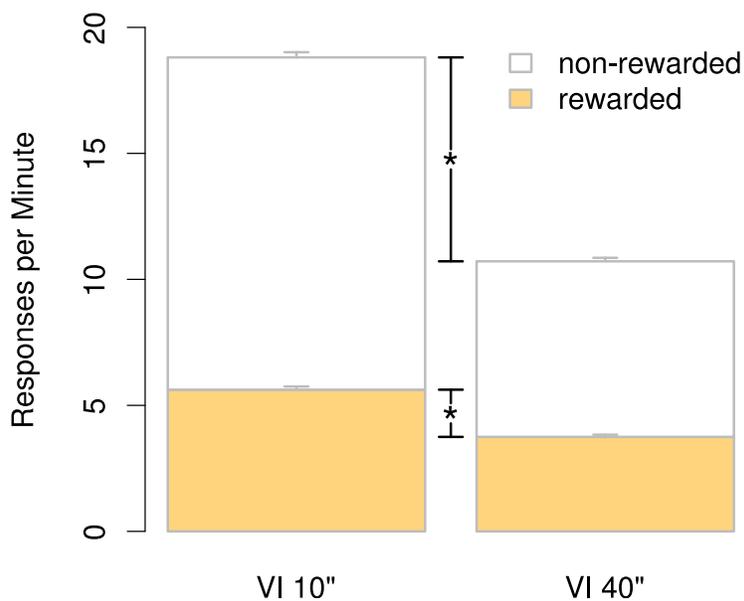


Figure 2.11: Rewarded and non-rewarded responses per minute during bias training. Rats pressed the more favorable VI 10" lever significantly more often than the other lever, and also received significantly more rewards from it. Error bars indicate standard error (SE); (*) $p < 0.05$.

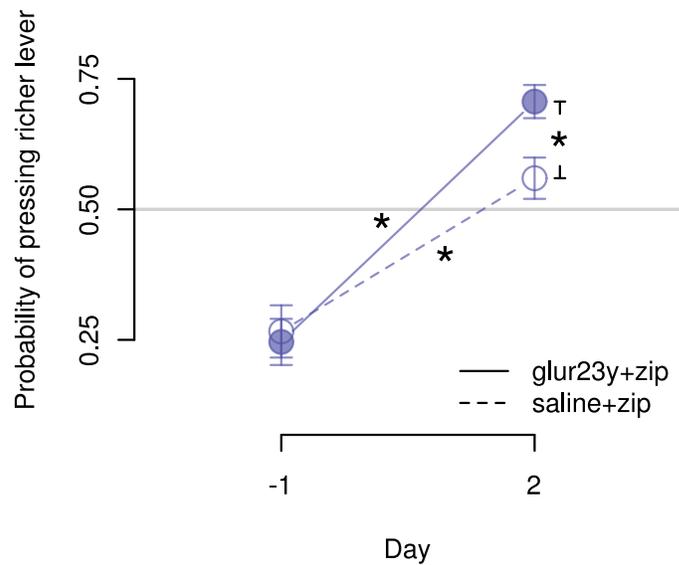


Figure 2.12: Probability of pressing the lever that was associated with the VI 10" during the reversal session. On the day before the reversal session, when this lever was associated with the VI 40", rats in both groups showed a similar low probability of pressing this lever. If saline was microinfused into the DMS one hour before ZIP, rats showed a decreased ability to adapt to the shift. If GluR2_{3Y} was microinfused into the DMS one hour before ZIP, rats did show an adaptation to the contingency reversal that was similar to the control groups in the previous experiment that received microinfusions of scr-ZIP. Error bars indicate standard error (SE); (*) $p < 0.05$.

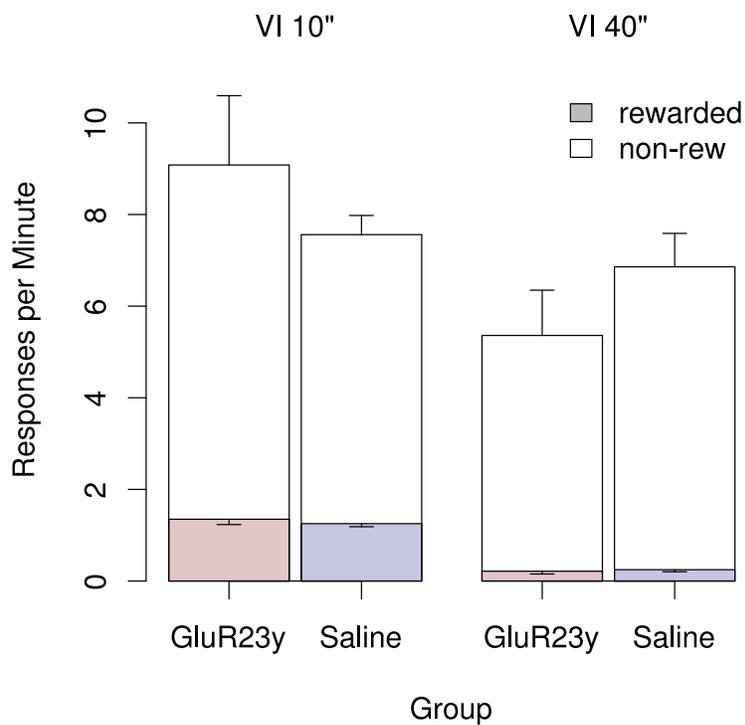


Figure 2.13: Rewarded and non-rewarded responses per minute at either lever. Rats with microinfusions of saline and ZIP into the DMS pressed both levers similarly often, despite the difference in ratio of rewarded to non-rewarded responses. Rats with microinfusions of GluR2_{3Y} and ZIP into the DMS increased responding on the lever with the VI 10", and reduced responding on the lever associated with the VI 40" schedule.

Chapter 3

Discussion

It has been established that the dorsomedial striatum (DMS) supports expectancy-driven behavior (Yin et al., 2005b; Kawagoe et al., 1998; Kimchi & Laubach, 2009b; Lau & Glimcher, 2008; O'Doherty et al., 2004) and that the dorsolateral striatum (DLS) supports habitual behavior (Yin et al., 2004). The motivation for this set of experiments was to determine whether the two areas actively maintain a long-term memory of task-relevant information that is critical for their separable contributions to an expectancy and a habit system (Shiflett & Balleine, 2011), or if they contributed to the storage of the relevant content in cortical areas that interact with these striatal areas (Pasupathy & Miller, 2005).

The results provided by our experiments are consistent with the hypothesis that these two regions of the striatum store information that is critical to performance in our contingency shift task.

We first demonstrated that infusions of the NMDA antagonist DL-AP5 into the DMS during testing impaired the rats ability to adapt to a contingency shift, but that infusions of DL-AP5 into the DLS enhanced their sensitivity to the contingency shift. These results are consistent with the established view that the DMS supports expectancy-driven behaviors and the DLS habitual behaviors. However, DL-AP5 is thought to block plasticity and the synaptic transmission in the striatum, so these results do not provide any insights into whether these areas store content long-term that differentially affects the rats' sensitivity to contingency changes.

In support of the hypothesis that the two dorsal striatal areas store task-relevant information, we found that infusions of ZIP into the DLS enhanced the rats' ability to adapt to the contingency shift, whereas infusions of ZIP into the DMS had the opposite effect. Infusions of GluR2_{3Y} one hour before ZIP into the

DMS rescued the rats' ability to adapt to the contingency shift. This result indicates that infusions of ZIP specifically targeted the ability of PKM ζ to regulate GluR2-dependent AMPA receptor trafficking, rather than causing an impaired sensitivity to contingency changes through a less specific effect.

Our experiments support the view that the DMS and DLS support task-relevant memories. However, they in no way rule out the view that other brain structures, in particular associated cortical regions, also store task-relevant information. Moreover, although our primary conclusion is reasonable, it must be viewed with caution. This is because we did not directly measure how AMPA receptor trafficking was affected by the acquisition and performance of the task, or by infusions of ZIP or GluR2_{3Y}. Fortunately, other studies that used the same methodology provide solid evidence that supports our interpretation.

Migues et al. (2010) found that inhibition of PKM ζ with ZIP in the amygdala impaired fear memory in rats, and that the magnitude of the impairment was proportional to the amount of reduction in the number of post-synaptic AMPA receptors. Simultaneously blocking the GluR2-dependent endocytosis of AMPA receptors with GluR2_{3Y} infusions abolished the behavioral deficit found after ZIP infusions, and the associated decrease of post-synaptic AMPA receptors. Using the same methodology, similar results were found for object location memory in dorsal hippocampus (Migues et al., 2010) and for drug reward memory in the core of the nucleus accumbens (Li et al., 2011). At the same time, over-expression of PKM ζ has been found to enhance memories long after they had been formed (Shema et al., 2011).

Given that these two striatal areas maintain a long-term memory of task-relevant information, it is reasonable to ask: What are the contents of these memories? This is a difficult question to answer, so one can only speculate about possibilities.

The DMS has previously been found to be involved in behavioral flexibility. Lesions of this area in primates are associated with perseverative impairments (Clarke, Robbins, & Roberts, 2008). In a behavioral reversal task, NMDA antagonism with DL-AP5 lead rats to regress back to the original behavior after an initially successful reversal (Palencia & Ragozzino, 2004). Similar results were obtained after temporarily inactivating this area (Ragozzino, Mohler, Prior, Palencia, & Rozman, 2009; Ragozzino, Jih, & Tzavos, 2002a; Ragozzino, Ragozzino, Mizumori, & Kesner, 2002b). Inactivation of this area or lesions have also

be found to abolish the sensitivity of rats to contingency degradation (Yin et al., 2005b).

But how does the DMS support behavioral flexibility? In order for a subject to rapidly adapt to contingency changes it needs to be able to (1) monitor performance to detect when a previously successful strategy is no longer successful and (2) derive a new estimate for the response outcome contingencies, so that it can then (3) reprogram behavior according to this new estimate (Gallistel, Mark, King, & Latham, 2001). Performance monitoring has been associated with the anterior cingulate cortex (Lapish et al., 2008), and determining contingencies with the prelimbic cortex (Balleine & Dickinson, 1998). Both of these frontal areas heavily interact with the DMS (Voorn et al., 2004). This analysis suggests that the DMS may store content that was needed to generate expectancies and evaluate them against the current demands of the task. Erasing this content would put the animal in the position of having to reassemble this information and thereby retard its ability to shift its behavior to the more appropriate lever.

To appreciate the potential content of the DLS, it is useful to remember that it is believed to support behaviors that are described as routine or habitual and not requiring extensive evaluation of the current situation (Balleine, Delgado, & Hikosaka, 2007; Pauli et al., 2009). Such behavior is thought to emerge as a consequence or repetition. Habitual behaviors are maladaptive when the learner confronts new contingencies. Thus, if the information that supports these maladaptive habits is erased while the information contained in the expectancy system is still available, one would expect to see a more rapid adaptation to the contingency shift.

In conclusion, our results support the generally accepted hypothesis that two regions of the striatum, the DMS and DLS, make different contributions to instrumental behavior and the ability to adapt to change. More importantly, by suppressing the activity of the memory maintenance protein, PKM ζ , we provided the first evidence that these regions do not simply enable storage structures outside of the striatum to influence behavior but themselves store information relevant to both behaving appropriately and adjusting to newly encountered behavioral contingencies.

Chapter 4

Materials and Methods

4.1 Subject and apparatus

Male Long-Evans rats (Harlan, Indianapolis, IN, U.S.A.) weighing between 325 - 349g at the beginning of the study served as subjects. Rats were housed individually in plastic tub cages in a temperature controlled room, with free access to food and water for the first week. The vivarium maintained a 12h light-dark cycle (lights on at 7:00 A.M.). Throughout the experiment subjects were restricted to maintain their weight at about 80 - 90% of their ad libitum weight with free access to water.

Training and testing took place in 8 operant chambers equipped with two response levers (Coulbourn Instruments), placed 5 cm to the left and right of a liquid dipper (Coulbourn Instruments) that delivered 2% reduced fat chocolate milk (Lucerne Foods, Pleasanton, CA, U.S.A.) as reward. Each chamber was housed within a sound- and light-resistant cabinet. A 3-W, 5-V house-light, mounted to the center of the ceiling provided illumination. Experimental protocols were reviewed and approved by the University of Colorado Institutional Animal Care and Use Committee (IACUC).

4.2 Surgery

Rats were anesthetized with Halothane and stereotaxically implanted with 26-gauge stainless steel guide cannulae (Plastics One) bilaterally into the posterior dorsomedial or dorsolateral striatum. Bregma was used as a reference. We used the following coordinates for bilateral implantation in dorsomedial striatum: anteriorposterior, -0.4 mm; mediolateral, $+/- 2.6$ mm; dorsoventral, 4.5 mm. We used the following

coordinates for bilateral implantation in dorsolateral striatum: anteriorposterior, + 0.7 mm; mediolateral, \pm 3.6 mm; dorsoventral, 5.0 mm.

4.3 Behavioral Procedure

4.3.1 Habituation

At the beginning of the experiment, a cup filled with 15 ml of chocolate milk was placed in home cages for up to two hours. This procedure was repeated daily until subjects had consumed all of the chocolate milk within 20 minutes.

4.3.2 Lever-press training

Rats were given daily, 45 min long, lever-press training sessions. Rats were trained on a variable-interval (VI) schedule of reinforcement. For each lever, the VI followed a uniform distribution. During the early stage of training all rats developed a response bias that required special training to ensure that they pressed the two levers at approximately the same rate. For this purpose a correction term was added to the VI of each lever to adjust for possible lever preferences: If a subject pressed one lever more often than the other, the difference in total lever-presses, since the beginning of the experiment, of this lever versus the other lever was added in seconds to the variable-interval. This increased both the minimum and the maximum of the uniform distribution. On the first day of training, or until a rat had pressed each lever at least 50 times (since the beginning of the experiment) reward was delivered at a VI 0.5 (min:0 s, max:1 s), followed by one session during which reward was delivered at a VI 5 schedule (min: 0 s, max: 10 s). On the following days, rats were trained on a VI 20" schedule, until they pressed each lever at least 100 times per session, and pressed both levers about equally often (less than 50 lever-presses difference between left and right lever).

4.3.3 Testing

After rats had reached these criteria cannulae were implanted. Rats were allowed to recover for 7 days, after which they were trained for several session with unchanged reinforcement schedules.

In the first experiment, in which DL-AP5 was injected later, rats were continued to be trained on concurrent VI 20" schedules again for two days, followed by another two days of training on the same schedule, but without the correction term for lever preferences. Rats were put through 2 two-day test cycles. On day 1 of each test cycle, the VI 20" schedule was associated with each lever. On day 2 of cycles 1 and 2 the schedules were shifted a less favorable VI 40" schedule was associated with the rats preferred lever and a more favorable VI 10" schedule was associated with the other lever. DL-AP5 was administered on day 2 of the first test cycle and vehicle was injected on day two of the second test cycle.

In the second and third experiment, in which we tested the role of PKM ζ on memory maintenance, rats were trained on an additional set of concurrent variable interval schedules. On the first day after post-surgical recovery, rats were again trained on concurrent VI 20" schedules without a correction term for lever preference. For the following sessions, the lever a rat had preferred during this session was assigned a less favorable VI 40" schedules, and the other lever a VI 10" schedule. Rats were trained on this schedule for. Microinfusions were performed on the first day after the last bias training session. 48h hours later, the schedules were reversed. A more favorable VI 10" schedule was associated with the lever that had been associated with a VI 40" during bias training, and vice versa for the other lever.

4.4 Microinfusions

We gently wrapped the rat in a soft towel, removed the obturator and inserted a 33 gauge microinjector (Plastics One) attached to polyethylene 50 (PE50) tubing through the indwelling guide cannula. The distal end of the PE50 tubing was attached to a 10-ml (Hamilton) syringe that was attached to a microinjection unit (model 5000; David Kopf Instruments) that accurately dispensed the desired volume at a rate of 0.5 μ l per minute. The microinjectors extended 0.5 mm into striatum beyond the tip of the guide cannulae. The injector remained connected for an additional minute to allow for drug to diffuse away from the tip of the

cannula.

4.5 Drugs

In the first experiment, DL-AP5, a selective NMDA antagonist provided by Tocris in powder form, was dissolved in a saline solution. We used a concentration of $4.5 \mu\text{g}/\mu\text{l}$, injecting $0.5 \mu\text{l}$ in 1 min for a total of $2.25 \mu\text{g}$ per side. Saline vehicle injections with the same parameters were used as control. In the second experiment we dissolved either the PKM ζ inhibitor ZIP ($30\text{nmol}/\text{side}/0.5\mu\text{l}$; Myr-SIYRRGARRWRKL; AnaSpec, catalog number 63361) or an inactive scrambled version of the peptide (scr-ZIP; $30\text{nmol}/\text{side}/0.5\mu\text{l}$; Myr-RLYRKRIWRSAGR; Anaspec, catalog number 63695) in saline (pH 7.0), and injected it into the DMS or the DLS.

In the third experiment we first injected either Tat-GluR2_{3Y} ($45\text{pmol}/\text{side}/0.5\mu\text{l}$; YGRKKRRQR-RRYKEGYNVYG; Anaspec, catalog number 64429) dissolved in saline (pH 7.0), or vehicle. One hour later we then injected the PKM ζ inhibitor ZIP ($30\text{nmol}/\text{side}/0.5\mu\text{l}$; Myr-SIYRRGARRWRKL; AnaSpec, catalog number 63361).

4.6 Histology

At the completion of the experiment, we anesthetized rats with pentobarbital (50 mg per kg body weight), decapitated them, removed their brains and froze the brains in cold isopentane. We cut coronal sections ($40 \mu\text{m}$ thick) through the striatum with a cryostat and mounted every third section. We stained sections with cresyl violet and examined them by light microscopy to visually verify the placement of the cannulae. Only rats with proper cannula placements were included in the experiment (Supplementary Figure 3).

4.7 Statistics

If the time between the current lever-press and the previous response was larger than the median response interval between lever-presses plus three times the standard deviation, the current lever-press was

removed from analysis.

To measure how well rats adapted to a contingency change, we calculated the relative probability of pressing the richer lever. Specifically, we divided the number of presses of the richer lever by the total number of lever presses within a time window of interest.

For the purpose of determining how well a rat had adapted to new contingencies we compared the relative probability of pressing a lever in the second half of the contingency shift session with the same relative probability during the second half of the last session before the contingency shift.

To determine the timecourse of adaptation we calculated the relative probability of pressing the richer lever between neighboring levels of progress. We calculated progress by dividing the current number of lever presses by the total number of lever presses by the end of the session. For calculating the progress through bias training we divided by the total number of lever-presses by the end of the bias training.

For statistical support we either used t-tests or linear mixed-effects models (nlme package for R). Linear mixed-effects models incorporate fixed effects, which are associated with repeatable levels of experimental factors, and random effects, which are associated with individual experimental units (e.g. subjects) drawn at random from a population (p. 3; Pinheiro & Bates, 2000). For planned comparisons we either used the Welch's t-test, which is an adaptation of Student's t-test intended for use with two samples having possible unequal variances, or the paired samples t-test for within-subject comparisons. T-statistics and p-values were rounded to the second decimal, if a p-value was smaller than 0.01, it was reported as 0.01.

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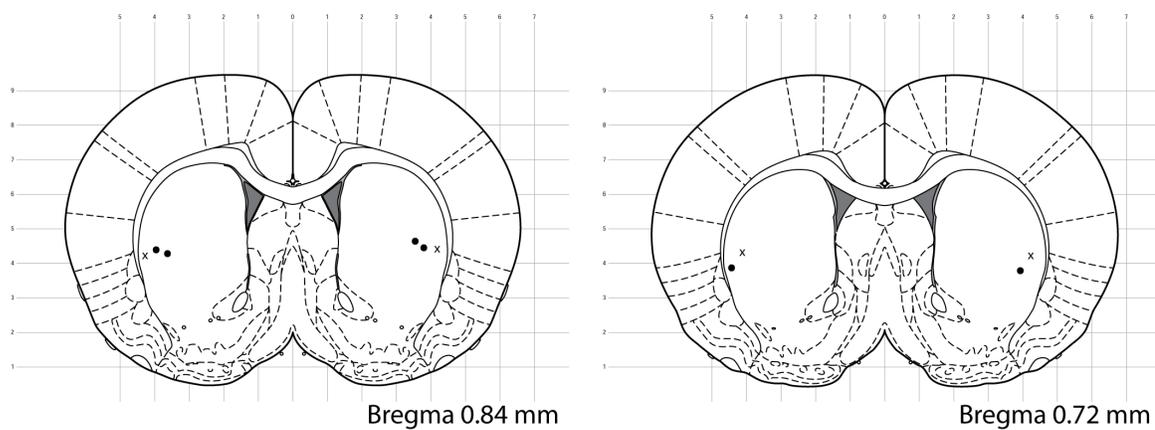
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Appendix A

Supplementary Material

A.1 Histology

Dorsolateral Striatum



Dorsomedial Striatum

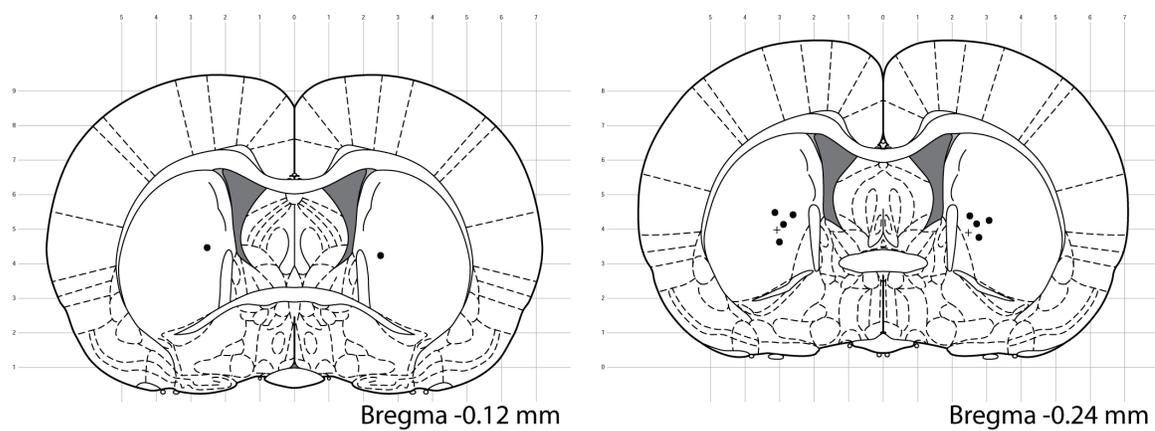


Figure A.1: Location of injector tips in the dlap5 experiment. Filled circles indicate locations at which DL-AP5 was injected. Crosses indicate locations at which saline was injected.

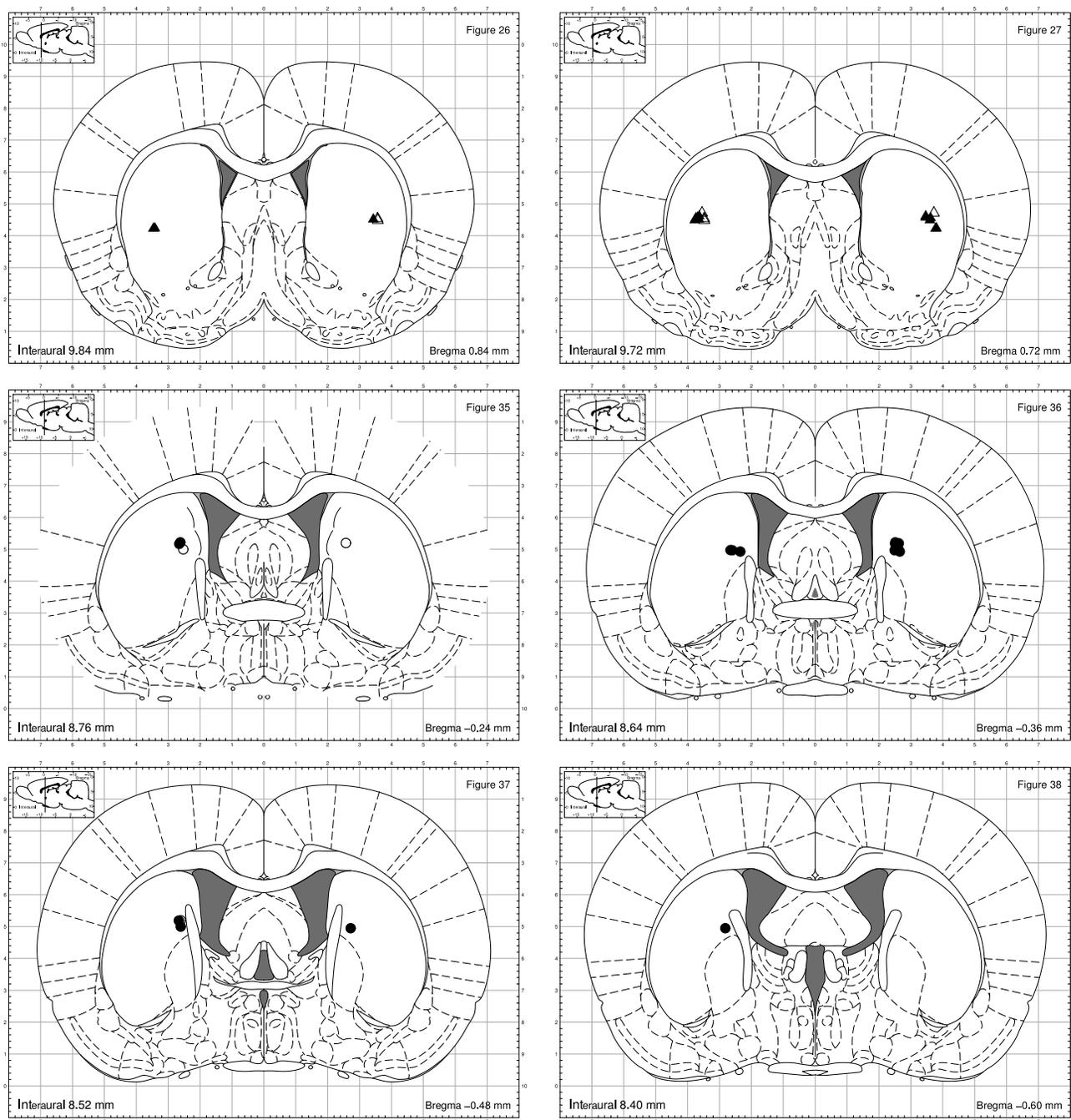


Figure A.2: Location of injector tips in first zip experiment. Filled circles indicate locations at which ZIP was injected. Empty circles indicate locations at which scr-ZIP was injected.

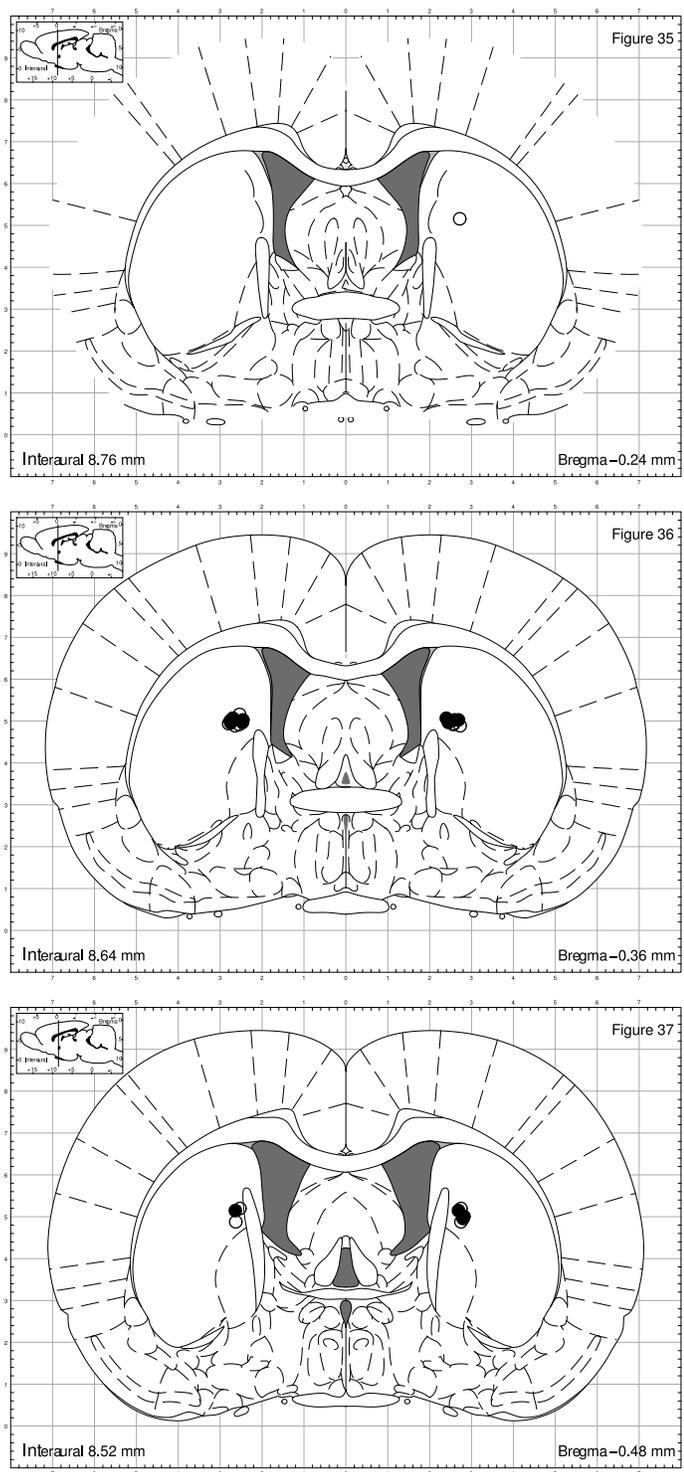


Figure A.3: Location of injector tips in second zip experiment. Filled circles indicate locations at which GluR2_{3Y} and zip were injected. Open circle indicate locations at which saline was injected before zip.

A.2 Response collection and reward delivery

Response collection and reward delivery were controlled by the PBN-lab (<http://grey.colorado.edu/CompCogNeuro/index.php/PBN-lab>) software that was developed by Wolfgang Pauli. PBN-lab is published under the GNU general public license (GPL).

Each chamber was connected via parallel port to a PC that controlled experimental contingencies. A web interface allowed the central control of all chambers, and the online monitoring of performance. To avoid experimenter errors, experimental parameters were determined automatically by the software, depending on the subject and its progress through the experiment.

Appendix B

Background

B.1 The role of DMS in behavioral flexibility

It is well established that the dorsomedial striatum (DMS) supports expectancy-driven behavior, and that the dorsolateral striatum (DLS) supports habitual behavior. We presented data that further supports the hypothesis that both areas maintain a long-term memory of task-relevant information. Specifically, these data suggest that the DLS maintains stimulus - response associations that result in perseverative behaviors incompatible with a rapid adaptation, while the DMS maintains information that enables it to support a rapid adaptation to new behavioral contingencies.

But what is the content that is stored in the DMS that enables it to support flexible behavior in this task? One approach to understanding this question is to recall that the DMS is associated with several prefrontal areas that support behavioral flexibility, most notably the prelimbic and the anterior cingulate cortex.

The first step in adapting to novel contingencies is to notice that the current behavioral strategy has become disadvantageous, the next is to determine the new contingencies and guide behavior according to them. Available data suggest that the anterior cingulate helps to determine if a current strategy is no longer appropriate, and that the prelimbic cortex is critical for determining the new contingencies.

One perspective is that the anterior cingulate cortex is critical for detecting that a strategy is no longer working by monitoring whether actions lead to expected outcomes. Some of the neurons in this area become particularly active when expected reward is omitted (Ito, Stuphorn, Brown, & Schall, 2003; Lapish et al.,

2008). Similarly, the BOLD response in human anterior cingulate (ACC) has also been consistent with this monitoring account (Holroyd & Coles, 2002; Carter, Braver, Barch, Botvinick, Noll, & Cohen, 1998; Rushworth, Walton, Kennerley, & Bannerman, 2004).

If our hypothesis is correct and the DMS, which is reciprocally connected with ACC, stores task-relevant information that is critical for detecting if actions do no longer lead to anticipated outcomes, a subset of DMS neurons should show a pattern of activity to correlates with activity in ACC. Consistent with our hypothesis, some neurons in the DMS also show an increased activity under conditions of increased uncertainty (Kimchi & Laubach, 2009a), and when performance is lower than expected (Thorn, Atallah, Howe, & Graybiel, 2010). These data indicate that ZIP infusions in the DMS might have prevented the DMS from using feedback information from the current task to enable the ACC to perform its role in performance monitoring. Because the DMS not only modulates activity in ACC, but also receives dense projections from this area, ZIP infusions probably also impaired the ability of the ACC-supported monitoring mechanism to exert control over behavior.

The other challenge imposed by the contingency shift in our experiment is to determine what the new contingencies are. Existing data suggest that the prelimbic cortex is involved in this aspect of the task. The prelimbic cortex is thought to be involved in determining response - outcome contingencies, and in choosing actions according to its estimate of how likely they are going to lead to reward (Balleine & Dickinson, 1998). This area of the rat medial frontal cortex is functionally similar to the vmPFC - mOFC in primates (Brown & Bowman, 2002; Uylings, Groenewegen, & Kolb, 2003). Lesion and inactivation studies in rats demonstrated that this area is critical for the detection of instrumental contingencies (Balleine & Dickinson, 1998; Dalley, Cardinal, & Robbins, 2004), and their changes (Ragozzino, 2007). By representing the value of different actions (de Wit, Corlett, Aitken, Dickinson, & Fletcher, 2009; Tanaka, Balleine, & O'Doherty, 2008; Noonan, Walton, Behrens, Sallet, Buckley, & Rushworth, 2010; Grabenhorst & Rolls, 2011), neurons of this region may guide choices accordingly (Seamans, Floresco, & Phillips, 1996). The DMS is also reciprocally connected with the prelimbic cortex. Similar to neurons in the prelimbic cortex, activity of neurons in the DMS of rats (Kawagoe et al., 1998; Lau & Glimcher, 2008; Kimchi & Laubach, 2009b),

and the BOLD response (O'Doherty et al., 2004) in the corresponding anterior caudate of humans has been found to reflect the expected value associated with actions.

The conclusions on the role of the interaction of the DMS with the ACC are similar to those for the prelimbic cortex. Infusions of ZIP into the DMS most likely affected both the ability of the DMS to enable prelimbic cortical function, and also the ability of prelimbic cortex to exert an influence over behavior.

B.2 PKM ζ maintains long-term memories

Even though the content of a particular memory depends on the underlying neuronal substrate, it is generally the result of long-term potentiation (LTP; Lømo, 1966), the persistent strengthening of synapses among neurons of an assembly that support this memory (Hughes, 1958). A strong stimulation of neurons can lead to an activation of glutamatergic AMPA receptors and NMDA receptors. The activation of NMDA receptors leads to an influx of calcium. This and other second messengers lead to an activation of various proteins that are active during learning, and for several hours after (Sanes & Lichtman, 1999). Particularly important for LTP are: Ca^{2+} /calmodium-dependent protein kinase II (CaMKII Lisman, Schulman, & Cline, 2002), mitogen-activated protein kinase (MAPK Kelleher et al., 2004), protein kinase A (PKA Nguyen & Woo, 2003), brain-derived neurotrophic factor (BDNF Patterson et al., 1992), and cyclic AMP-responsive element-binding protein (CREB Tully, 1991). However, if the action of these proteins is inhibited after an initial time window closes, there is no disruption of already stored memories (Sanes & Lichtman, 1999; Sacktor, 2011). These negative results lead to the preliminary conclusion that memories are stored in the remodeled morphology of synapses, which then shared the same molecules as synapses formed during development (Kandel, 2001).

However, how can it be then that memories can last a lifetime, but almost all the molecules in our bodies turnover in a matter of days (Crick, 1984). In fact, the potentiation of a synapse activates homeostatic processes that increase the endocytosis and therefore the molecular turnover of AMPA receptors (Migues et al., 2010). A candidate, self-perpetuating enzymatic molecular mechanism for the maintenance of long-term memory that addresses this often over-looked aspect of human memory might have been found in the

constitutively active protein kinase C (PKC) isoform, protein kinase M ζ (PKM ζ). This PKM ζ maintains long-term memory traces by continually regulating trafficking of AMPA receptors, which are essential for fast excitatory synaptic transmission. Even though PKM ζ seems to solve the molecular turnover problem for AMPA receptors of memory maintenance, how does it itself escape the molecular turnover?

Most PKC isoforms are only active as long as second messengers (e.g. CA⁺⁺) bind to their regulatory domain to cause a conformational change, that activates the kinase. Because second messengers are rapidly eliminated, the activity of most PKC isoforms fades within minutes (Sacktor, 2011). In contrast, unlike most PKC isoforms, PKM ζ only consists of a catalytic domain, but no regulatory domain. Without this regulatory domain, the catalytic domain is constitutively active (Hernandez, Blace, Crary, Serrano, Leitges, Libien, Weinstein, Tcherapanov, & Sacktor, 2003), and may be self-perpetuating, by phosphorylating PIN1 (protein interacting with NIMA1 Sacktor, 2010), a prolyl isomerase which blocks the translation of mRNA under basal conditions.

In which manner does PKM ζ reconfigure AMPA receptor trafficking to maintain an increased number of AMPA receptors in the synapse? Through interactions with the GluR2 subunits of AMPA receptors, NSF maintains a basal number of AMPARs in the synapse (Lüscher, Xia, Beattie, Carroll, von Zastrow, Malenka, & Nicoll, 1999). PICK1 (protein interacting with C kinase 1) on the other hand participates in the endocytic removal of AMPA receptors from synapses and maintains a pool of these receptors outside the synapse (Hanley, Khatri, Hanson, & Ziff, 2002). Even though the exact mechanism is not known, PKM ζ seems to functionally enhance the ability of NSF to release GluR2-containing receptors from PICK1 (Sacktor, 2011), maybe by binding to PICK1 itself (Yao, Kelly, Sajikumar, Serrano, Tian, Bergold, Frey, & Sacktor, 2008). It has been suggested that this interaction of NSF with PICK1 has two effects: (1) it moves AMPA receptors that are maintained in these extrasynaptic pools into the synapse, and reduces the PICK1-mediated endocytosis of GluR2 containing AMPA receptors (Sacktor, 2011).

A second mechanism has been proposed through which NSF prevents GluR2-containing AMPA receptors endocytosis. Like PICK1, guanine-nucleotide exchange factor brefeldin-resistant Arf-GEF 2 protein (BRAG2; also known as IQSEC1) also binds to the tyrosine-rich C-terminal of the GluR2-subunit.

This activates the GTPase Arf6 (Scholz, Berberich, Rathgeber, Kolleker, Köhr, & Kornau, 2010), which then recruits adapter protein complex 2 (AP2), a key mediator of endocytosis at the plasma membrane (Collingridge, Peineau, Howland, & Wang, 2010). The binding area of the AP2 overlaps with that of NSF (Lee, Liu, Wang, & Sheng, 2002; Collingridge, Isaac, & Wang, 2004). The synthetic peptide GluR2_{3Y} (⁸⁶⁹YKEGYNVYG⁸⁷⁷) has been found to block BRAG2 mediated LTD (Ahmadian, Ju, Liu, Wyszynski, Lee, Dunah, Taghibiglou, Wang, Lu, Wong, Sheng, & Wang, 2004; Yu et al., 2008), supposedly by mimicking the tyrosine-rich C-terminal of the GluR2 subunit (Ahmadian et al., 2004), reducing binding of BRAG2 to this region of the GluR2 subunit (Sacktor, 2011).

It has also been shown that postsynaptic perfusion of GluR2_{3Y} blocks the LTP reversal found after ZIP injections (Migues et al., 2010). Furthermore, injecting ZIP one day after fear conditioning in rats has been found to lead to amnesia (Migues et al., 2010; Parsons & Davis, 2011). If GluR2_{3Y} was fused to the cell membrane transduction domain of the HIV 1 Tat protein for cell permeability (Frankel & Pabo, 1988; Green & Loewenstein, 1988; Dietz & Bähr, 2004), and injected 1 hour before ZIP, however, it prevented this amnesia and the endocytosis of GluR2 containing AMPA receptors (Migues et al., 2010). Analogous results have been found for the maintenance of memories of relapse-evoking reward cues after injections of GluR2_{3Y} and ZIP into the NAc (Li et al., 2011), and object location memory in the dorsal hippocampus (Migues et al., 2010). Even though PKM ζ is abundant in the striatum (Naik, Benedikz, Hernandez, Libien, Hrabe, Valsamis, Dow-Edwards, Osman, & Sacktor, 2000), to our knowledge it has not been investigated whether PKM ζ is involved in maintaining dorsal striatal memories associated with instrumental behavior (Li et al., 2011).

These results do not only show that PKM ζ regulates trafficking of GluR2 containing AMPA receptors, they also demonstrate that ZIP specifically targets the action of PKM ζ on this subunit (Migues et al., 2010; Sacktor, 2011). The combination of ZIP and GluR2_{3Y} is an excellent experimental tool to investigate the maintenance of long-term memories, because neither GluR2_{3Y} (Brebner et al., 2005; Migues et al., 2010; Yu et al., 2008) nor ZIP injections affect non-potentiated synapses (Ling, Benardo, Serrano, Blace, Kelly, Crary, & Sacktor, 2002; Serrano, Yao, & Sacktor, 2005; Sajikumar, Navakkode, Sacktor, & Frey, 2005;

Pastalkova et al., 2006; Madroñal, Gruart, Sacktor, & Delgado-García, 2010).

If the maintenance of synaptic potentiation depends on the presence and absence of PKM ζ , how is the right level of PKM ζ activity maintained at a specific synapse? One recent proposal is that the persistent trafficking of GluR2_{3Y} receptors itself might maintain the kinase at appropriate synaptic sites (Sacktor, 2011). The results of simultaneous injections of GluR2_{3Y} and ZIP provide evidence for this auto-tagging model. If the mechanisms that maintain PKM ζ at the appropriate synapses are separate from the regulation of GluR2-dependent AMPA receptor trafficking, memory-loss should occur, unless GluR2_{3Y} plays an unknown role outside its effect on blocking GluR2 receptor endocytosis.