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The brain-derived neurotrophic factor receptor TrkB is critical for the acquisition but not expression of conditioned incentive value

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Abstract

Stimuli paired with reward acquire incentive properties that are important for many aspects of motivated behavior, such as feeding and drug-seeking. Here we used a novel chemical–genetic strategy to determine the role of the brain-derived neurotrophic factor (BDNF) receptor TrkB, known to be critical to many aspects of neural development and plasticity, during acquisition and expression of positive incentive value by a cue paired with food. We assessed that cue's learned incentive value in a conditioned reinforcement task, in which its ability to reinforce instrumental responding later, in the absence of food itself, was examined. In *TrkB*^{F616A} knock-in mice, TrkB kinase activity was suppressed by administering the TrkB inhibitor 1NMPP1 during the period of initial cue incentive learning only (i.e. Pavlovian training), during nose-poke conditioned reinforcement testing only, during both phases, or during neither phase. All mice acquired cue–food associations as indexed by approach responses. However, *TrkB*^{F616A} mice that received 1NMPP1 during initial cue incentive learning failed to show conditioned reinforcement of nose-poking, regardless of their treatment in testing, whereas administration of 1NMMP1 only during the testing phase had no effect. The effects of 1NMPP1 administration were due to inhibition of TrkB^{F616A}, because the performance of wild-type mice was unaffected by administration of the compound during either phase. These data indicate that BDNF or NT4 signaling through TrkB receptors is required for the acquisition of positive incentive value, but is not needed for the expression of previously acquired incentive value in the reinforcement of instrumental behavior.

Introduction

Recent findings have identified neural processes that underlie emotional and motivational aspects of food-rewarded learning (Everitt & Robbins, 2005). Initially, neutral stimuli paired with food acquire incentive properties, such that they may modulate feeding (Petrovich *et al.*, 2005, 2007), influence the performance of other learned responses (Holland & Gallagher, 2003), and serve as conditioned reinforcers (Mead & Stephens, 2003). This last function, by which animals learn to perform instrumental responses that earn presentation of cues that had previously been paired with reward, is especially interesting because conditioned reinforcers often serve important roles in establishing and maintaining behavioral control in the absence of primary reinforcers such as food (Mackintosh, 1974). A number of studies have indicated that conditioned reinforcement depends on the function of several forebrain regions, including the basolateral amygdala, orbitofrontal cortex, and ventral striatal nucleus accumbens (Cador *et al.*, 1989; Parkinson *et al.*, 1999; Pears *et al.*, 2003). Considerably less is known about the molecular mechanisms that mediate this form of positive incentive learning. Since brain-derived neurotrophic factor (BDNF) is essential for certain forms of learning, as well as processes that underlie cellular plasticity associated with learning, it is a candidate mediator for positive incentive learning.

BDNF is a member of a family of neurotrophic growth factors, the neurotrophins, that are critically involved in neuronal development, survival, and synaptic plasticity (Klein *et al.*, 1991, 1993; Korte *et al.*, 1995; Minichiello *et al.*, 2002). The BDNF receptor, TrkB, has been implicated in learning conditioned fear (Rattiner *et al.*, 2004). However, elucidation of most functions of BDNF–TrkB signaling in the mature nervous system has been hampered by limitations of genetically modified animals; most TrkB null mice die perinatally (Klein *et al.*, 1993; Linnarsson *et al.*, 1997). In the present study, we used an experimental model that circumvents this problem and enables assessment of TrkB function in the adult, mature nervous system. This approach combines the specificity of gene targeting and the temporal control and reversibility of pharmacological manipulations to test the role of BDNF–TrkB signaling in a conditioned reinforcement task. The small molecule inhibitor 1NMPP1 blocks TrkB kinase activity in mice harboring a TrkB F616A mutation (*TrkB*^{F616A}), but not in

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wild-type mice (Chen *et al.*, 2005). Because application of 1NMPP1 to *TrkB*^{F616A} mice allows rapid inhibition of TrkB kinase activity, TrkB signaling could be regulated during different phases of the conditioned reinforcement task. This feature of the chemical–genetic strategy enables independent assessments of the contribution of TrkB function during the initial learning phase, when a cue paired with food acquires incentive value, and during the subsequent test phase, in which the performance of a novel instrumental response is reinforced by that cue. Initially, *TrkB*^{F616A} mice were trained on a Pavlovian discrimination, whereby presentations of one auditory cue resulted in sucrose reinforcement [reinforced conditioned stimulus (CS^+)], and a second auditory cue was non-reinforced [non-reinforced conditioned stimulus (CS^-)]. We later evaluated incentive learning by testing whether the mice would learn to nose-poke to earn presentations of CS^+ in the absence of food.

Materials and methods

Generation of mice

The *TrkB*^{F616A} mice were generated as previously described (Chen *et al.*, 2005). Mice were backcrossed into a C57BL/6 background for two generations. The *TrkB*^{F616A} mice were bred as homozygous mating pairs.

Chemical synthesis and administration of 1NMPP1

1NMPP1 was synthesized as described previously (Chen *et al.*, 2005). Fresh injection solution was made daily; 125 μL of either pure dimethylsulfoxide (DMSO) or 100 mM 1NMPP1 DMSO solution was added to 1.5 mL of filtered saline and 2.5% Tween-20 solution. Mice received daily 150 μL injections between 15:00 h and 17:00 h, throughout Pavlovian training and conditioned reinforcement testing, as well as during the 5 days prior to each of those treatment phases. Throughout the experiment, *TrkB*^{F616A} and wild-type mice in Group vehicle-vehicle received daily injections of DMSO, and *TrkB*^{F616A} and wild-type mice in Group 1NMPP1-1NMPP1 received daily injections of 1NMPP1. *TrkB*^{F616A} mice in Group 1NMPP1-vehicle received daily injections of 1NMPP1 during the Pavlovian conditioning phase (and the five previous days) and DMSO during the conditioned reinforcement test phase (and the five previous days); *TrkB*^{F616A} mice in Group vehicle-1NMPP1 received the two injection types in the opposite order. In parallel with injections, DMSO or 1NMPP1 was added to the water supply. One hundred and sixty microliters of either DMSO or 1NMPP1 added to 2 mL of filtered saline and 2.5% Tween-20 solution was then added to 500 mL of distilled water. This water supply was replaced every third day.

Behavioral methods

Subjects

Behavioral testing was conducted with 48 age-matched *TrkB*^{F616A} mice and 16 C57/BL6J wild-type mice (Jackson Laboratory, Bar Harbor, ME, USA). The *TrkB*^{F616A} mice were bred at the Johns Hopkins University School of Medicine and transferred to the Neurogenetics and Behavior Center, Johns Hopkins University, for behavioral testing at 6 months of age. Prior to testing, the *TrkB*^{F616A} mice were separated into four groups: 1NMPP1-1NMPP1 (n = 13); 1NMMP1-vehicle (n = 14); vehicle-1NMMP1 (n = 9); and vehicle-vehicle (n = 12). Wild-type mice were separated into two groups, 1NMMP1-1NMMP1 (n = 8) and vehicle-vehicle (n = 8), that received experimental treatment identical to that received by *TrkB*^{F616A} mice, with similar group designations. Mice were housed three or four to a cage under a 12 h light/dark cycle (lights on at 07:00 h to 19:00 h). Food deprivation began 5 days before the start of testing and continued throughout training. All mice were food deprived to 85% of their *ab libitum* weights by limiting access to a single daily meal. Behavioral training and testing was completed in the light cycle between 09:00 h and 13:00 h. All experiments were conducted according to the National Institutes of Health's Guide for the Care and Use of Laboratory Animals, and the protocols were approved by the Johns Hopkins University Animal Care and Use Committee.

Apparatus

Behavioral training was conducted in eight individual chambers (length, 53 cm; width, 35 cm; height, 35 cm) with aluminum front and back walls, clear polycarbonate sides, and a floor made of 17.8 mm stainless steel rods spaced 0.5 cm apart (Med Associates, St Albans, VT, USA). A food cup was recessed in the center of one end wall into which 0.1 mL of liquid reward could be delivered. A vacuum was attached to the bottom of the food cup, which could be released via an attached solenoid. An infrared photocell placed inside the food cup monitored time spent and number of entries into the food cup. An audio generator that could emit either a 3 kHz tone or white noise (amplitude set 5 dB above background; approximately 80 dB) was mounted on the outside of the chamber on the wall opposite the food cup. Chamber illumination was provided by a 28 V, 100 mA house light mounted on the inside wall of the sound-attenuating chamber. During the conditioned reinforcement testing phase, the chambers were fitted with two nose-poke manipulanda, each 12 mm in diameter, and located at identical heights on the left and right sides of the food cup. Each nose-poke contained a yellow stimulus LED located at the rear of the recessed hole and a photobeam sensor to monitor nose-poke entries. An IBM-compatible computer equipped with MED-PC software (Med Associates) controlled and recorded all stimuli and responses.

Pavlovian conditioning procedures

The mice were first trained to consume rewards delivered to the food cup. Rewards (0.1 mL of 10% w/v sucrose solution) were delivered on a random-time 30 s schedule, in each of two daily 40 min training sessions. Next, the mice received a single Pavlovian training session each day, for a total of 14 sessions. Each session was approximately 30 min long and consisted of 12 presentations, 10 s long, each of a 3 kHz tone and of a white noise, with a variable intertrial interval of 120 s. For half the mice in each group, sucrose was delivered to the food cup for the final 3 s of the tone (CS^+) but not for the noise (CS^-). For the remaining mice, the noise served as CS^+ and the tone served as CS^-. Any sucrose remaining at the end of CS^+ presentation was automatically vacuumed out at that time. CS^+ and CS^- trials were intermixed in pseudorandom sequences determined by the computer. The response measure reported was the time spent in the food cup during a CS, expressed as a percentage of the 10 s CS duration.

Conditioned reinforcement test

After 5 days in which the drug regimen was altered in Group vehicle-1NMPP1 and Group 1NMPP1-vehicle, mice received a single 40 min conditioned reinforcement test session. During this test, nose-poke ports were made available on both sides of the food cup. For half the mice in each group, each nose-poke to the left port resulted in the brief (3 s) presentation of the tone cue, and each right nose-poke response produced a 3 s noise presentation. Nose-pokes made during a cue presentation were recorded but had no programmed consequences. For

the remaining mice, the response–stimulus contingencies were reversed. The Pavlovian conditioning histories of tone and noise (as CS^+ or CS^-) were also completely counterbalanced with respect to left or right nose-poke responses. If CS^+ acquired the ability to serve as a conditioned reinforcer, then more nose-pokes that produced CS^+ would be performed than nose-pokes that produced CS^-.

Results

Pavlovian conditioning

We used $TrkB^{F616A}$ mice and 1NMPP1, a selective inhibitor of $TrkB^{F616A}$ but not wild-type TrkB, to assess the role of TrkB signaling during the acquisition and expression of positive incentive learning.

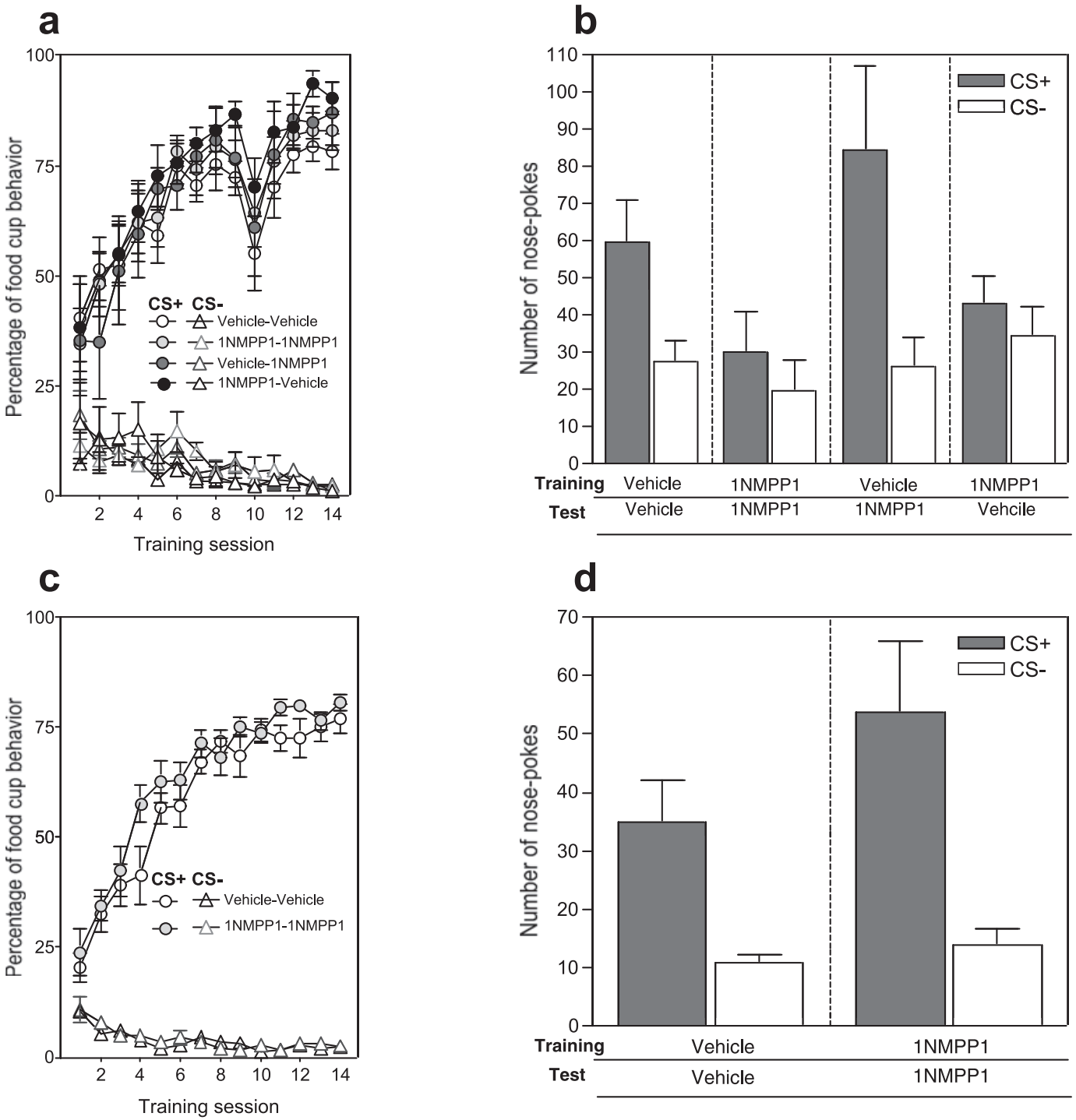

FIG. 1. Performance of 1NMPP1-treated or vehicle-treated $TrkB^{F616A}$ and wild-type mice in Pavlovian training and conditioned reinforcement testing. (a and c) Mean ± SEM percentage of time spent in the food cup during the 10-s presentations of the rewarded CS^+ (circles) and non-rewarded CS^- (triangles) for $TrkB^{F616A}$ (a) and wild-type mice (c). (b and d) Mean + SEM total nose-poke responses during the 40-min conditioned reinforcement test for 3 s presentations of the previously rewarded cue (gray bars) and non-rewarded cue (white bars) for $TrkB^{F616A}$ (b) and wild-type (d) mice.

Administration of 1NMPP1 to wild-type mice provides a control for specificity of the pharmacological treatment. Treatment of *TrkB*^{F616A} (Fig. 1a) and wild-type (Fig. 1c) mice with 1NMPP1 during Pavlovian training had no effect on the acquisition of conditioned responding directed to the primary reward of food; all mice increased their response to the food cup during CS^+ but not during CS^-. Thus, all mice acquired an association between CS^+ and sucrose. In *TrkB*^{F616A} mice, a receptor status (active or inactive) × cue (CS^+ or CS^-) × session ANOVA showed a main effect of cue ($F_{1,46} = 546.2$, $P < 0.0001$) and a cue × session interaction ($F_{13,598} = 45.8$, $P < 0.0001$), but no effect of receptor status ($F_{1,46} = 1.16$, $P = 0.29$) or receptor status × cue interaction ($F_{1,46} = 0.24$, $P = 0.625$). Similarly, the analysis from the wild-type conditioning data revealed a main effect of cue ($F_{1,14} = 1196.94$, $P < 0.0001$) and a cue × session interaction ($F_{13,182} = 76.4$, $P < 0.0001$), but no effect of 1NMPP1 treatment ($F_{1,14} = 1.68$, $P = 0.22$) or 1NMPP1 treatment × cue interaction ($F_{1,14} = 1.53$, $P = 0.237$). Collectively, these results indicate that all groups of mice readily acquired the Pavlovian discrimination during the conditioning phase. In addition, measures of general activity and consumption of the reward itself (Table 1) showed no significant differences among the groups (F-values < 1.29, P-values > 0.33).

Conditioned reinforcement test

The data of primary interest, instrumental nose-poke responding in the conditioned reinforcement test, are presented separately for *TrkB*^{F616A} (Fig. 1b) and wild-type (Fig. 1d) mice. *TrkB*^{F616A} mice that received vehicle during Pavlovian training (Group vehicle-vehicle and Group vehicle-1NMPP1) showed a conditioned reinforcement effect; these animals performed more nose-pokes that earned CS^+ than nose-pokes that earned CS^-. In contrast, *TrkB*^{F616A} mice that received 1NMPP1 during Pavlovian training (Group 1NMPP1-vehicle and Group 1NMPP1-1NMPP1) were no more likely to perform the CS^+-associated poke than the CS^--associated one. Thus, pairings of CS^+ with food while TrkB activity was suppressed failed to establish positive incentive value for that CS^+, as expressed by a failure at test to preferentially respond for presentations of this cue. Interestingly, once this incentive value was ascribed to CS^+, administration of 1NMPP1 during the conditioned reinforcement test had no effect on the ability of the cue to promote new instrumental learning. Finally, the observation that 1NMPP1 administration had no effect on performance in wild-type mice supports the claim that the effects of 1NMPP1 administration in the *TrkB*^{F616A} mice were due specifically to suppression of TrkB kinase activity, rather than a non-specific or off-target action of 1NMPP1.

This impression was confirmed by ANOVA conducted on the nose-poke tests for *TrkB*^{F616A} and wild-type mice. For *TrkB*^{F616A} mice, ANOVA of test responding with variables of receptor status in training (top line of abscissa label), receptor status in test (bottom line of abscissa label) and response (earning CS^+ or CS^-) showed a significant interaction of TrkB status in training with response ($F_{1,44} = 10.46$, $P = 0.002$), but not of receptor status in test with response ($F_{1,44} = 1.59$, $P = 0.214$). Individual comparisons showed significantly more responses to earn CS^+ than to earn CS^- in Group vehicle-vehicle ($F_{1,44} = 8.73$, $P = 0.005$) and Group vehicle-1NMPP1 ($F_{1,44} = 21.54$, $P < 0.001$), but not in Group 1NMPP1-1NMPP1 ($F_{1,44} = 0.98$, $P = 0.326$) or Group 1NMPP1-vehicle ($F_{1,44} = 0.75$, $P = 0.392$). A similar analysis conducted on the wild-type mice revealed that, unlike *TrkB*^{F616A} mice, wild-type controls showed no effect of drug treatment on conditioned reinforcement. ANOVA of test responding with variables of treatment and response (earning CS^+ or CS^-) showed a main effect of response ($F_{1,14} = 22.57$, $P < 0.001$) but no effects of treatment ($F_{1,14} = 0.289$, $P = 0.60$) or treatment × response interaction ($F_{1,14} = 0.006$, $P = 0.94$).

Although the differences in overall nose-poke levels among the groups were within the range of normal variation, the wild-type mice appeared to show less nose-poking overall than the two groups of *TrkB*^{F616A} mice that showed successful conditioned reinforcement (Group vehicle-vehicle and Group vehicle-1NMPP1). Despite these apparent differences in overall response rate, the mutant and wild-type mice showed comparable magnitudes of conditioned reinforcement, as indexed by discrimination ratio scores, the ratio of responding for CS^+ to total responding (CS^+ plus CS^- responding). A ratio that exceeds 0.5 indicates greater responding for CS^+ than for CS^-. There were no significant differences in discrimination ratio among *TrkB*^{F616A} Groups vehicle-vehicle (mean ± SEM = 0.68 ± 0.06) and vehicle-1NMPP1 (0.71 ± 0.05). Additionally there were no significant differences between these groups and wild-type Groups vehicle-vehicle (0.72 ± 0.04) or 1NMPP1-1NMPP1 (0.78 ± 0.03); largest $t_{(18)} = 1.37$, ps < 0.18. By contrast, TrkB^{F616A} Group 1NMPP1-vehicle (0.53 ± 0.04) and Group 1NMPP1-1NMPP1 (0.58 ± 0.07) each displayed significantly lower discrimination ratios than all other groups; smallest $t_{(23)} = 2.21$, ps < 0.03). This analysis provides further support for our assertion that the deficits in conditioned reinforcement were due to inactivation of TrkB^{F616A} at the time of CS food learning.

Finally, it is notable that, consistent with the results of phase 1, food cup entries during the nose-poke test phase (in which no food was delivered, but nose-poke contingent deliveries of CS^+ might be expected to evoke conditioned food cup responses) did not differ among the groups (data not shown, $F < 1$).

TABLE 1. Responding during Pavlovian training

	Group/condition					
	Vehicle-Vehicle (TrkBF616A)	1NMPP1-1NMPP1 (TrkBF616A)	Vehicle-1NMPP1 (TrkBF616A)	1NMPP1-vehicle (TrkBF616A)	Vehicle-vehicle (C57BL/6J)	1NMPP1-1NMPP1 (C57BL/6J)
CS^+ reward consumption, lick totals						
First session	182 ± 98	133 ± 31	277 ± 75	185 ± 38	245 ± 37	308 ± 109
Last session	643 ± 31	608 ± 90	633 ± 107	629 ± 69	710 ± 63	770 ± 74
CS^+ activity counts						
First session	33 ± 6	26 ± 4	24 ± 6	33 ± 8	49 ± 8	38 ± 5
Last session	42 ± 5	35 ± 7	25 ± 12	36 ± 8	32 ± 6	30 ± 8

Lick totals and activity counts are mean ± SEM (per 14 trials each of 10 s duration).

Discussion

In this study, administration of 1NMPP1 to either wild-type or *TrkB*^{F616A} mice had no effect on the acquisition of simple Pavlovian discrimination, in which mice were trained to associate a particular cue with reward. Interestingly, in *TrkB*^{F616A} mice, but not wild-type mice, 1NMPP1 treatment during the training period blocked the acquisition of positive incentive value by CS^+, as revealed at test by their failure to learn to perform nose-pokes to earn presentations of the previously rewarded cue. By contrast, 1NMPP1 administration during the conditioned reinforcement test alone had no effect in any of the mice. The absence of an effect of 1NMPP1 treatment of wild-type mice during the training period demonstrates that this deficit was due to 1NMPP1-induced suppression of TrkB function in the *TrkB*^{F616A} mice. Taken together, these results indicate that TrkB signaling is critical in adult mice for the acquisition of positive incentive or reward value by a cue paired with food, but not for the acquisition of simple food-directed approach responses to that cue. Furthermore, once a cue has acquired reward value, TrkB signaling is apparently not necessary for the cue to serve as a reinforcer for subsequent instrumental responding.

In the central nervous system, BNDF binding to TrkB results in autophosphorlyation of a range of downstream signaling cascades, which include the transcriptional expression of the BDNF gene and a range of other plasticity-related genes, including *α-CaMKII* and *GAP-43*, as well as the immediate early genes FOS and JUN (Koponen *et al.*, 2004). BDNF also regulates the activity of a number of neurotransmitter systems, including dopaminergic (Guillin *et al.*, 2001; Do *et al.*, 2007), glutamatergic (Kim *et al.*, 2006; Caldeira *et al.*, 2007) and serotonergic (Rumajogee *et al.*, 2002) pathways. Furthermore, BDNF has emerged as a key regulator of synaptic plasticity in the adult nervous system. Both genetic and pharmacological approaches have implicated BDNF–TrkB signaling during multiple, distinct stages of hippocampal long-term potentiation, including the induction and maintenance phases (Korte *et al.*, 1995; Kang *et al.*, 1997; Minichiello *et al.*, 2002; Chen *et al.*, 2005), and recent findings point to a role for TrkB in the development of synaptic consolidation during long-term potentiation (Tanaka *et al.*, 2008). Mechanistically, BDNF–TrkB signaling controls synaptic transmission through regulation of transcriptional events as well as dendritic translation of mRNAs (Aakalu *et al.*, 2001; Patterson *et al.*, 2001).

Although there is general consensus that BDNF–TrkB signaling can modulate synaptic transmission and plasticity, the role of this signaling module in behaving animals has been difficult to discern. One concern with approaches using mice harboring null or conditional mutations in either *BDNF* or *TrkB* to address the behavioral roles of the BDNF signaling module is that behavioral deficits observed in those mutant mice may reflect either developmental requirements or adult functions of BDNF–TrkB signaling. The present study, using *TrkB*^{F616A} mice and a chemical–genetic approach, circumvents this problem and has enabled us to determine the contribution of BDNF–TrkB signaling during different phases of a behavioral paradigm.

Given its widespread function, our findings that TrkB inactivation during training prevented the acquisition of incentive value for CS^+ (Fig. 1b), but left acquisition of learned responding directed to the primary food reinforcer intact (Fig. 1a), may seem surprising. Notably, we also found no differences among the groups in general activity or in lickometer measures of consumption of the sucrose reinforcer itself (Table 1). However, this dissociation between incentive learning and other products of learning procedures is consistent with considerable previous research on the critical neural circuitry for incentive learning. For instance, animals with damage to either the orbitofrontal cortex, the ventral striatal nucleus accumbens or the basolateral amygdala show normal acquisition of food cup approach responses to CSs that predict food delivery, but they cannot subsequently use those CSs as conditioned reinforcers in new learning [for review, see Baxter & Murray (2002)]. Moreover, mice with a targeted deletion of the GluR1 AMPA receptor subunit display similar deficits, specific to conditioned reinforcement (Mead & Stephens, 2003). The current findings provide the first demonstration that TrkB is involved in this selective aspect of reward learning, and indicate that an alteration in TrkB function may contribute to disruption to one or more of these systems/pathways. The use of intracranial infusions of 1NMPP1 into particular brain regions (e.g. Kaneko *et al.*, 2008) in *TrkB*^{F616A} mice, and pharmacological techniques targeted to specific BDNF–TrkB signaling systems, should prove informative.

Expression of TrkB has been implicated in the development of behavioral disorders such as obesity and addiction (Castren, 2004; Corominas *et al.*, 2007). With respect to obesity, TrkB is expressed in the ventromedial nucleus of the hypothalamus, where it functions to regulate energy homeostasis and food intake (Kernie *et al.*, 2000; Anubhuti, 2006; Unger *et al.*, 2007; Tsao *et al.*, 2008). Alternatively, TrkB expression in the hippocampus plays a role in the development of conditioned place preference and drug sensitization (Shen *et al.*, 2006), and its expression in the ventral striatum is associated with persistent cocaine-seeking behaviors and heightened sensitivity to relapse in both rats and mice (Graham *et al.*, 2007). Thus, characterizing the role of this receptor in motivational learning processes, such as conditioned reinforcement, may have important implications for the long-term therapeutic treatment of behavioral disorders.

Our results indicate that TrkB function is critically involved in the acquisition of positive conditioned incentive value for environmental cues. The ability of reward-associated cues to influence behavior is particularly relevant for understanding disorders of behavioral control, such as drug abuse and addiction. Drug-related cues (e.g. drug paraphernalia and contexts of drug use) often play important roles in addicts' drug-searching strategies (Everitt & Robbins, 2005), and in relapse in abstaining addicts (Crombag & Shaham, 2002). Further investigation of the role of TrkB in learning processes will provide novel insights into the molecular mechanisms underlying both adaptive and maladaptive motivational learning. In particular, the use of techniques to rapidly and reversibly inactivate TrkB in adult mice, as in the present study, should be of further value in this endeavor.

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Abbreviations

BDNF, brain-derived neurotrophic factor; CS, conditioned stimulus; DMSO, dimethylsulfoxide.

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