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Overexpression of Neuronal RNA-binding protein HuD increases reward induced reinstatement of an instrumental response

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Abstract

The neuronal RNA-binding protein HuD is involved in synaptic plasticity and the molecular mechanisms of learning and memory. Previously, we have shown that HuD is upregulated after both spatial and addiction-associated forms of learning, such as conditioned place preference. However, what role HuD plays in non-drug dependent learning and memory is not fully understood. In order to elucidate the role that HuD plays in non-drug appetitive behavior, we assessed mice over-expressing HuD (HuD_{OE}) throughout the forebrain on the acquisition of an instrumental response for a non-sucrose food reward utilizing a touch-screen paradigm. Next, we examined whether HuD level would alter the extinction or reward-induced reinstatement of responding. We found that HuD_{OE} acquired and extinguished the instrumental response at rates similar to control littermates with no significant alterations in secondary measures of motor behavior or motivation. However, HuD_{OE} reinstated their responding for food reward at rates significantly higher than control animals after a brief presentation of reward. These results suggest that HuD positively regulates the reinstatement of natural reward seeking and supports the role of HuD in forms of learning and memory associated with seeking of appetitive rewards.

Keywords

RNA binding protein; HuD; post-transcriptional regulation; reward seeking

Introduction

Post-transcriptional regulation affects gene expression through many mechanisms including the control of mRNA stability or the ability of recently transcribed mRNA to be translated into functional protein [1]. Given its simultaneous influence over mRNA stability and protein translation, it is no surprise that post-transcriptional regulation within specific brain regions has been implicated in numerous behaviors [2–10]. HuD is one such neuronal expressed RNA binding protein (RBP; [11]) that is known to stabilize specific target mRNAs through direct interaction with specific consensus sequences within the 3'UTRs

[12–15]. Through this mechanism, HuD has been found to directly regulate the stability and translation of two mRNAs heavily implicated in synaptic plasticity, *Camk2a* and *Bdnf*. HuD is localized to hippocampal neuron dendrites [16], where it has been implicated in synaptic tagging of *Camk2a* mRNA [15].

HuD has also been associated with hippocampal-dependent learning [17,18] and seizures [19]. In addition, neuronal activity is known to regulate HuD expression, localization and association with plasticity associated mRNAs such as *Homer1a* and *Camk2a* [20]. Recent studies indicate that HuD also plays a role in translation [21]. The regulation of translation by HuD is in turn modulated by upstream factors such as PKC, which has been found to increase local translation of BDNF by phosphorylation of HuD [22]. Altogether, these results indicate that HuD expression and localization may play a role in protein synthesis-dependent forms of plasticity.

Since many learning paradigms have been found to be protein synthesis-dependent, RBPs that influence translation, such as HuD, may mediate performance on these tasks [23]. Instrumental learning is one such procedure thought to recruit protein-synthesis dependent mechanisms [24] and the reinstatement of an instrumental response after extinction has been proposed to model relapse in drug addiction, suggesting that this disorder may also be protein synthesis-dependent [25–27]. In addition, reinstatement of responding for non-drug reward is a valuable tool to understand the normal functioning of these systems. Although progress has been made in the transcriptional and epigenetic regulation of these behaviors [28,29], little is known regarding the role of the HuD RBP in acquiring, extinguishing, or reinstating responses for reward. Previously, we have shown that a transgenic mouse that robustly overexpresses HuD (HuD_{OE}) throughout the forebrain has alterations in learning and memory [18,19,30]. Here, we sought to elucidate the role of the HuD RBP in reward related learning behaviors. We assessed HuD_{OE} and control littermates on the acquisition, extinction, and reward induced reinstatement of an instrumental response for a food reward utilizing a touch-screen paradigm previously shown to distinguish between reward seeking behavior in different inbred strains of mice [26].

Methods

Subjects

Transgenic mice overexpressing HuD under the *Camk2a* promoter were generated and backcrossed to a C57BL/6J background for >20 generations [18]. Mice were housed in groupings of 2–4 per cage in a temperature and humidity-controlled vivarium under a reverse 12 h light/dark cycle (lights off 0800 h) and tested during the dark phase. Male HuD overexpressing (HuD_{OE}) and control littermates (8 control; 10 mutant) were tested on acquisition, extinction and reinstatement in a touch-screen operant chamber as previously described [26,33–35]. Mice were aged 12 weeks at the onset of behavioral testing. All experimental procedures were performed in accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals and were approved by the University of New Mexico Health Sciences Center Institutional Animal Care and Use Committee.

Operant apparatus

All operant behavior was conducted in a chamber measuring $21.6 \times 17.8 \times 12.7$ cm (model # ENV-307W, Med Associates, St. Albans, VT, USA) housed within a sound- and light-attenuating box (Med Associates) as previously described. The standard grid floor of the chamber was covered with a solid acrylic plate to facilitate ambulation. A pellet dispenser delivering non-sucrose 14 mg dustless pellets (#F05684, BioServ, Frenchtown, NJ, USA) into a magazine, a house-light, tone generator and ultra-sensitive lever were located at one end of the chamber. At the opposite end of the chamber there was a touch-sensitive screen (Conclusive Solutions, Sawbridgeworth, UK) covered by a black acrylic aperture plate allowing two 2×5 cm touch areas separated by 0.5 cm and located at a height of 6.5 cm from the floor of the chamber. Stimulus presentation in the response windows and touches were controlled and recorded by the KLimbic Software Package (Conclusive Solutions, Sawbridgeworth, U.K.).

Pretraining

Mice were first slowly reduced and then maintained at 85% free-feeding body weight. Prior to testing, mice were acclimated to food reward by provision of ~10 pellets/mouse in the home cage for 3-5 days followed by habituation to the operant chamber for 30 min with pellets available in the magazine. Mice retrieving 10 pellets within 30 min were moved to lever press training where mice obtained 1 reward by pressing an ultrasensitive lever within the chamber. Reward delivery was concomitant with the compound presentation of 2 cues, consisting of a 1-sec 65 dB auditory tone and illumination of the food magazine, that were designed to serve as explicit secondary reinforcers. Mice were required to collect each reward, measured by magazine beam-break, before another reward could be gained by lever-press. Mice pressing and collecting 30 rewards in under 30 minutes were moved to the acquisition phase.

Acquisition of an instrumental response

For the acquisition phase, lever press led to the presentation of a two white square stimuli (1×2.8 cm² white square per window) in both response windows that remained on the screen until a response was made (Figure 1A). A touch of either stimulus led to the delivery of a single food reward concomitant with the two secondary reinforcers (1-sec tone and magazine light). Mice were required to initiate and respond to either of 2 stimuli over 30 trials (5 sec ITI). Mice were trained on acquisition until they reached a criterion of 30 responses within 15 min on each of 5 consecutive sessions.

Extinction training

Assessment of extinction began on the session immediately following acquisition criterion. During extinction, the same visual stimuli (1×2.8 cm² white square per window) were automatically presented (lever inactive) for 10 sec or until a touch was made at either response window (10 sec ITI; Figure 1B). A touch at either stimuli during presentation produced neither food US reinforcement nor the conditioned secondary reinforcers. Stimuli were presented for 30 trials during each extinction session. Mice continued on extinction

training until mice met a criterion of 77% omissions (no-response to stimuli during presentations) per session for 2 consecutive sessions.

Brief-reward induced reinstatement

Mice were assessed for reinstatement of instrumental responding on the session immediately following extinction. During reinstatement, the visual stimuli automatically appeared in both response windows for 10 sec. or until a touch was made at either location (10 sec ITI; Figure 1C). To induce reinstatement of responding, reward was delivered concomitant with secondary reinforcers during the first 6 presentation trials regardless of stimulus touch. Rewarded trials were followed by 54 additional trials during which stimuli were automatically presented but no reward or secondary reinforcers were delivered regardless of response. Reinstatement of instrumental responding was determined by calculating the difference in responding during the reinstatement session versus responding during the final day of extinction.

Statistical Analysis

The effect of genotype on sessions to criterion, trials omitted, latency to response and latency to reward retrieval where applicable was analyzed via Student's t-test. The effect of genotype on the response made, omissions, latency to respond and latency to retrieve reward were analyzed using analysis of variance (ANOVA), with repeated measures for trial session across problem.

Results

During pre-training, all mice of both genotypes underwent habituation to consuming food reward in the touch-screen chamber in 1 session. No significant differences were seen on the total sessions to acquire lever-press behavior for a non-sucrose food reward ($t(16)=.36$; $p=.72$; $HuD_{OE}=4.4\pm0.9$; $control=5.1\pm1.4$). Mice of both genotypes rapidly acquired the simple instrumental response to a visual stimulus in ~20 sessions. Analysis of performance across acquisition trials found that all mice rapidly decreased their time to complete sessions (main effect of session $F_{19,114}=11.86$, $p=0.001$) but HuD_{OE} mice did not differ from controls (main effect of genotype: $F_{1,114}=0.22$, $p=0.88$) and there was no interaction between session and genotype ($F_{19,114}=0.67$, $p=0.85$; Figure 2A). Similarly, latency from initiation to response rapidly decreased across sessions (main effect of session $F_{19,114}=6.19$, $p=0.01$) but did not differ across genotypes (main effect of genotype $F_{1,14}=0.25$, $p=0.63$) with no significant interaction ($F_{19,114}=0.55$, $p=0.93$). Similar results were seen for latency to receive food reward (main effect of session $F_{1,114}=11.90$, $p=0.01$; main effect of genotype $F_{19,114}=0.14$, $p=0.72$; interaction $F_{1,14}=0.76$, $p=0.74$). No significant differences were found in number of sessions to acquire the instrumental response to a high level of responding ($t(16)=0.63$, $p=0.54$; Figure 2B) or total average latency to respond to stimuli across sessions ($t(16)=1.68$, $p=0.11$) or retrieve reward ($t(16)=0.88$, $p=0.38$; Figure 2C). Similarly, no differences were seen between genotypes in trials omitted during early acquisition sessions ($t(16)=0.32$, $p=0.76$; $HuD_{OE}=11.7\pm4.8$, $control=9.8\pm4.0$).

When primary food and secondary tone and magazine light reinforcers were removed during extinction training, all mice rapidly decreased responding to stimuli. Analysis of performance across extinction sessions found that all mice increased number of omitted trials (main effect of session ($F_{7,14}=4.87$, $p=0.01$) but HuD_{OE} mice did not differ from controls (main effect of genotype: ($F_{7,14}=0.320$, $p=0.62$; Figure 3A) and there was no significant interaction of genotype $\times$ behavior ($F_{7,14}=0.74$, $p=0.64$). Similarly, latency to touch on response trials increased across sessions (main effect of session $F_{7,14} = 3.48$, $p=0.02$) but did not differ across genotypes (main effect of genotype $F_{7,14} = 0.78$, $p=0.47$). Analysis of total sessions to extinguish the instrumental response to criterion did not reveal an effect of genotype ($t(15)=1.59$, $p=0.13$; Figure 3B) and HuD_{OE} mice did not differ from control on average reaction time when mice responded to non-rewarded stimuli ($t(16)=0.07$, $p=0.94$) or to explore the magazine when reward was withheld ($t(16)=1.07$, $p=0.30$; Figure 3C).

When mice underwent testing on reinstatement via brief exposure to the food reward and secondary reinforcers HuD_{OE} mice showed response patterns that differed significantly from control mice. HuD_{OE} mice showed similar rates of responses during the last session of extinction HuD_{OE}= $5.2 \pm .49$; control= $4.9 \pm .48$; ($t(16)=0.52$, $p=.60$). Similarly, HuD_{OE} mice responded to the first 6 primed sessions of the reinstatement at similar rates to controls ($t(16)=-0.47$, $p=.64$), with no significant differences between latency to respond to primed stimuli ($t(16)=0.67$, $p=0.52$) or retrieve reward during the first 6 sessions ($t(16)=0.04$, $p=0.96$). In contrast, HuD_{OE} mice responded to the following unrewarded trials at significantly higher rates than control ($t(16)=2.21$, $p=0.04$; Figure 4A). Analysis of responding relative to the last extinction session revealed significant increases in reinstated versus littermate controls ($t(16)=2.75$, $p=0.01$; Figure 4B). Analysis of the latency to respond to stimuli during reinstatement found that genotypes did not significantly differ ($t(16)=-0.17$, $p=0.87$) nor did groups differ on latency to check the magazine for reward after responses ($t(16)=0.09$, $p=0.93$; Figure 4C).

Discussion

In the current study, we have found that HuD specifically regulates reward induced reinstatement of an instrumental response, as acquisition and extinction were not significantly altered in HuD_{OE} mice. Analysis of secondary measures revealed no differences in response or reward latencies, indicating that motor function or motivation for food reward was not responsible for these differences. Together, these findings suggest that HuD plays a specific role in reinstating food reward seeking behavior following extinction.

Previously, we have shown that HuD_{OE} mice display alterations in learning and memory dependent behavior associated with spatial and reward-related learning [18,30]. In particular, HuD_{OE} mice have significant increased acquisition of preference for a non-contingent drug reward during conditioned place preference (CPP) [30]. While CPP measures differ from the paradigms used here in significant ways, CPP is hypothesized to have phases of acquisition, extinction, and reinstatement similar to those utilized in the current study suggesting HuD may play a common role in these different learning behaviors [25]. While we specifically examined the effects of HuD manipulation on seeking of non-drug food reward,

maintenance and relapse of drug seeking has been modeled using extinction and reinstatement tasks analogous to those used here [25]. This is particularly important as HuD target mRNAs have been suggested to play a role in the reinstatement of an instrumental response for drug, but have not been examined in food reward [31,32]. Drug craving and seeking behaviors are hypothesized to be mediated by corticolimbic circuits [36,37] and seeking of food reward may recruit similar circuitry, albeit in a less direct stimulatory capacity as drugs of abuse [38]. Based on effects of HuD overexpression on reinstatement of responding for food rewards here, and drug preference in previous studies, we hypothesize that HuD plays a role in regulating reward seeking across both drug and food appetitive rewards via involvement in a common behavioral circuit.

The specific circuits associated with reinstatement of an extinguished response differs depending on the eliciting factor (e.g. stress, cue, reward), but dopaminergic input from the ventral tegmental area (VTA) to the prelimbic cortex (PrL), nucleus accumbens core (NAcC), and ventral pallidum (VP) appear to play a central role regardless of factor [39,40]. Pharmacological or optogenetic inhibition of these subregions blocks reinstatement by stressors, presentation of reward-associated cues or brief exposure to the reward. Basolateral amygdala (BLA) also plays a specific role in reward-associated context and cue reinstatement, via inputs from lateral cortical regions including the orbitofrontal cortex and its subsequent inputs to NAc and PrL [37]. Previous work had primarily characterized the involvement of HuD in behaviors that recruit the hippocampus or NAc [16,18,30]. While the paradigms used in the current experiment likely recruit these areas as well, the reinstatement effects seen in the current study suggest HuD may regulate other circuits beyond the hippocampus or the NAc.

While our data suggests that HuD may regulate seeking for multiple appetitive rewards, further study is needed to understand specific mechanisms. However, one likely candidate is via HuD-dependent stabilization of target mRNAs. As mentioned previously, past studies have shown that HuD acts on CaMKII α and BDNF mRNA, which are both involved in several forms of learning and memory during the development of drug seeking behaviors [31,32]. One behavior that these HuD targets were studied in was the cue-reinstatement of drug-seeking behavior. For example, BDNF within the NAc, amygdala, and possibly VTA has been implicated as the molecular factor involved in incubation for drug craving, an effect that seems to potentiate reinstatement of cocaine intake [43]. Moreover, BDNF may play a role in cue-induced cocaine seeking, as attenuation of NAc Shell (NAcSh) BDNF signaling on withdrawal day 90 after self-administration led to decreased cue-induced cocaine seeking [44]. This stimulatory effect upon cue-induced cocaine seeking appears to be heavily region specific, as mPFC BDNF infusion or NAc overexpression may inhibit cocaine seeking [44–47]. Finally, NAcSh CaMKII, another mRNA target of HuD, has been found to be necessary for reinstatement [32]. Two of its major targets have been implicated in reinstatement of drug seeking, suggesting that HuD may play a common role in the increased reinstatement of reward seeking, whether the appetitive reward is drug-based or more naturalistic.

While there is evidence that HuD mRNA targets may play a role in a parallel instrumental learning behavior, very little is known on their role in food reward studies. In particular, little work has been done to examine the role that either of these targets may play in instrumental

learning for non-drug reward. However, there is evidence that Ser-831 on the GluR1, a CaMKII α specific phosphorylation site, may be important for the retrieval of instrumental learning for an food reward [41]. Combined with evidence that HuD regulates CaMKII activity in other circumstances [15,20,30,42], it is possible that HuD regulatory pathways involving CaMKII α action may mediate these behaviors, although more studies are required to establish this link.

In conclusion, data from the current study suggest that HuD specifically plays a role in reinstatement, but not acquisition or extinction, of an instrumental response for food reward. In turn, this suggests that regulation of mRNA stability and protein translation mediated by this RBP may be involved in control of seeking regardless of reward type. Further, these data suggest that post-transcriptional regulation is involved in regulating whether to engage in instrumental behaviors and underline the utility of these tasks in examining control over reward seeking.

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Figure 1: Touch-screen acquisition, extinction and reinstatement paradigm.

(A) During acquisition, mice are trained to respond *via* lever press to receive a food pellet reward concomitant with magazine light and tone by touching 1 of 2 white square stimuli on a touch-sensitive screen. (B) During extinction stimuli appear automatically on screen every 10 seconds and touch responses do not produce reward or secondary reinforcers (C). For reinstatement, mice are primed for 6 trials via automatic delivery of reward and secondary reinforcers. Reinstatement of responding is measured over the following 54 unrewarded trials.

Figure 2: Overexpression of HuD does not alter rate of acquisition of an instrumental response for food reward.

(A) All mice significantly decreased time to complete acquisition sessions and HuD^{OE} mice showed rates similar to controls. (B) Genotypes did not differ on total sessions to reach acquisition criterion (C) HuD^{OE} did not significantly alter total average latency to respond to the stimuli or retrieve non-sucrose food reward after a response during acquisition. (Data are Means ± SEM).

Figure 3: HuD^{OE} mice show normal extinction of instrumental response.

(A) HuD^{OE} and control mice showed similar rates of extinction measured by total trials omitted across sessions (B) No differences were seen between genotypes on sessions to reach extinction criterion (C) HuD^{OE} had similar latencies to touch stimuli when they responded to non-rewarded trials and did not differ on latency to check the reward magazine after a response. (Data are Means $\pm$ SEM).

Figure 4: HuD overexpression significantly increases responding during reward induced reinstatement.

(A) HuD^{OE} mice did not respond at higher rates than controls during the brief (6 trial) rewarded priming trials but responded significantly more during the remainder of the session. (B) Responding during reinstatement was significantly higher in HuD^{OE} versus controls compared to the number of responses on the preceding session of extinction. (C) HuD^{OE} did not differ from controls on latency to responds or check the reward magazine either during primed or unrewarded trials. (* $p < .01$ versus control animals; Data are Means $\pm$ SEM).