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Narp knockout mice show normal reactivity to novelty but attenuated recovery from neophobia

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Abstract

Narp knockout (KO) mice demonstrate cognitive inflexibility and addictive behavior, which are associated with abnormal reactivity to a novel stimulus. To assess reactivity to novelty, we tested Narp KO and wild-type (WT) mice on a neophobia procedure. Both Narp KO and WT mice showed a similar decrease in consumption upon initial exposure to a novel flavor, but Narp KO mice did not increase consumption with subsequent exposures to the novel flavor like the WT mice. Therefore, Narp KO mice do not have abnormal reactivity to novelty but show deficits in adapting behavior to reflect the updated value of a stimulus.

Keywords

Narp; neophobia; extinction; novelty; addiction; recovery from neophobia

Neuronal activity-regulated pentraxin, or Narp, is a secreted immediate early gene product that clusters AMPA receptors (AMPARs; O'Brien et al. 1999). Narp-positive neurons are present in many limbic and cortical regions, including the prefrontal cortex, hippocampus, hypothalamus, and amygdala (Reti et al. 2002a; Reti et al. 2002b; Reti and Baraban 2003; Johnson et al. 2010). In previous studies we found that Narp knockout (KO) mice acquire instrumental and Pavlovian learning normally (Johnson et al. 2007; Crombag et al. 2009), but show behavioral inflexibility after learning has occurred. Specifically, Narp KO mice are

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deficient in a food reinforcer devaluation procedure (Johnson et al. 2007) and have impaired extinction of morphine conditioned place preference (CPP, Crombag et al. 2009).

Both cognitive inflexibility and addictive behavior have been linked to abnormal reactivity to a novel stimulus (Barcelo et al. 2002; Flagel et al. 2013). In addition, Narp levels in the prefrontal cortex, a brain area implicated in cognitive flexibility and addiction (Van den Oever et al. 2010; Kesner and Churchwell 2011), have been shown to correlate with locomotor activity in a novel environment (Lu et al. 2002). Taken together, these findings suggest that Narp KO mice may have abnormal reactivity to novelty.

To assess whether Narp KO mice differ in their reaction to novelty compared to wild-type (WT) mice, Narp KO mice and their WT littermates were tested on a task designed to assess initial reactivity to novelty (neophobia) and recovery from neophobia. Typically, mice display neophobia, or the innate response to inhibit intake of a novel food upon first presentation. Upon repeated presentations of the novel food in the absence of negative consequences, mice will gradually increase their intake over time or show recovery from neophobia.

We assessed neophobia and recovery from neophobia using 8–10-week-old male Narp KO (n=12) and WT (n=12) littermates obtained by breeding Narp heterozygotes that had been backcrossed 12 times to the C57BL/6 strain (Johnson et al. 2007). Mice were kept on a 12-hr-light/dark cycle with lights on from 07:00–19:00. Mice were separated and single housed for one week prior to starting neophobia, and all stages of the neophobia procedure were performed in the home cage. Prior to the start of the neophobia procedure, freely available food (standard rodent chow) and water were removed.

The neophobia procedure consisted of an acclimation phase and an experimental phase (Fig 1). The liquid diet (hereafter referred to as the non-flavored diet) used for the acclimation and experimental phase was a 23% w/w powdered rodent diet (item 7551; Quality Lab Products, Inc, Elkridge, MD) dissolved in water using a blender (Oster). For acclimation to this non-flavored diet, mice were given ad libitum access to it for 7 days. On day 1–8 of the experimental phase, all mice were given a 2 hr exposure (from 12:00–14:00) to the non-flavored diet and a 1 hr exposure (from 16:00–17:00) to either the non-flavored diet (baseline days 1–2, 4, 6–7) or to a flavored diet (Test days 3, 5, 8). The flavored diet consisted of the non-flavored diet with the addition of 3% w/w Sanka instant decaffeinated coffee (Maxwell House).

Mice were weighed daily at 11:00 during the acclimation and experimental phases. During the experimental phase, mice were supplemented with an additional food pellet (standard rodent chow) when necessary to keep weight at 90% of free feeding weight. If necessary, the supplemental pellet was given at 17:30 following the 1 hr exposure to the liquid diet and removed prior to weighing at 11:00 the next day. Neither the weight of the mice nor the number of mice needing a supplemental pellet differed between genotypes. Bottles were weighed daily at 11:00 during acclimation and before and after all diet presentations during the experimental phase. During the 1 hr session on baseline day 7, one bottle leaked so this data point was excluded.

Consumption is reported in grams of liquid diet consumed or as a suppression ratio. For calculating the suppression ratio, grams of flavored liquid diet consumed during the Test was divided by the grams consumed during the Test plus the grams consumed during the 1 hr presentation of the non-flavored diet on the baseline day prior to the Test (grams consumed during Test/(grams consumed during Test + grams consumed during prior baseline day)). A suppression ratio of 0.5 indicates equivalent consumption of the flavored diet on a Test day and the non-flavored diet on the previous baseline day. Consumption was

analyzed with either paired t-tests or with repeated measures ANOVA followed by Fisher LSD post-hoc tests.

No differences were found between Narp KO and WT mice in the consumption of the non-flavored diet during either acclimation (Fig 2A) or during the first 2-hr exposure to the non-flavored diet on days 1–8 of the experimental phase (Fig 2B). During the second 1 hr exposure to the diet on days 1–8 (Fig 3A), Narp KO mice did not differ from WT mice in consumption during baseline days. During Test 1, both Narp KO and WT mice showed a similar decrease in consumption. Repeated measures ANOVA across baseline day 2 and Test 1 showed an effect of Session ($F_{(1,22)}$=123.13, p<0.001) but no effect of Genotype nor a Genotype × Session interaction (p>0.1) indicating that both Narp KO and WT mice showed a similar neophobic response to the initial exposure of the flavored diet. However, on Tests 2 and 3, Narp KO mice showed an attenuated increase in consumption compared to WT mice, indicating attenuated recovery from neophobia. Repeated measures ANOVA across Test 1–3 showed an effect of Genotype ($F_{(1,22)}$=9.72, p=0.005), Test Session ($F_{(2,44)}$=28.38, p<0.001) and a Genotype × Test Session interaction ($F_{(2,44)}$=5.39, p=0.008). A Fisher LSD post-hoc test showed significant differences between Narp KO and WT mice on Test 2 (p=0.005) and Test 3 (p<0.001). When test data were calculated as suppression ratios (Fig 3B), a repeated measures ANOVA across Test 1–3 showed an effect of Genotype ($F_{(1,21)}$=8.24, p=0.009), Test Session ($F_{(2,42)}$=19.91, p<0.001) and a Genotype × Test Session interaction ($F_{(2,42)}$=3.62, p=0.035), again indicating attenuated recovery from neophobia in the Narp KO. A Fisher LSD post-hoc test was significant for Test 2 (p=0.015) and Test 3 (p<0.001) but not for Test 1 (p>0.1).

Since Narp KO mice did not differ from WT mice in their initial neophobia response, we tested another group of Narp KO (n=15) and WT (n=15) littermates using a weaker flavored decaffeinated coffee solution (1.5%) as the 3% decaffeinated solution could have been so strong as to mask any differences on Test 1. When tested with the weaker decaffeinated coffee solution, both WT and KOs still showed similar neophobic responses (Fig 4). Repeated measures ANOVA across baseline day 2 and Test 1 showed an effect of Session ($F_{(1,28)}$=8.78, p=0.006) but not Genotype or Genotype × Test Session interaction (p>0.1; Fig 4A). Narp KO and WT mice did not differ in their suppression ratio on Test 1 ($t_{(14)}$=0.47, p>0.1; Fig 4B).

Our results suggest that Narp KO mice do not show altered reactivity to novelty as they do not show altered intake of a novel solution upon initial presentation when compared to the WT mice. This finding is consistent with data showing that Narp KO mice do not differ from WT mice in their locomotor response to a novel environment (Pacchioni et al. 2009). However, Narp KO mice do show attenuated recovery from neophobia and continue to avoid the novel solution with subsequent presentations rather than increase their consumption like that of WT mice. This attenuated recovery from neophobia in the Narp KO mice is consistent with our prior data reporting that Narp KO mice show inflexibility during a reinforcer devaluation task and during morphine CPP (Johnson et al. 2007; Crombag et al. 2009). In the reinforcer devaluation and morphine CPP tasks, Narp KO mice are able to learn new associations initially, but cannot modify or update these associations once they are presented with new information from the environment. In the neophobia procedure, mice are presented with a novel taste which may be accompanied by negative consequences. Once they consume a small amount of the solution and do not become ill, they are unable to modify their behavior by taking into account that the taste is safe. Thus, they do not demonstrate the normal increase in consumption of the solution shown by WT mice.

Another possible interpretation is that Narp KO mice may have memory deficits and may not remember the initial presentation of the novel-flavored solution. It is notable that aging

rats also show attenuated recovery from neophobia (Gallagher and Burwell 1989). The relationship between Narp levels and aging has not been elucidated. It is unlikely that our finding is due to altered taste processing by Narp KO mice, as they do not show differences in reactivity to either a 3% or a 1.5% decaffeinated coffee solution. However, a thorough comparison of the palatability of the solution in Narp KO and WT mice was not performed.

Finally, it is notable that addictive behavior has been associated with increased novelty seeking, although this relationship is complex and contradictory evidence exists (Erb and Parker 1994; Antoniou et al. 2004; Mitchell et al. 2005; Pelloux et al. 2006). Although Narp KO mice show addiction-like behavior in the morphine CPP paradigm, they do not show increased consumption of a novel solution in the neophobia procedure and continue to avoid a novel solution with subsequent presentations. Thus, extinction deficits in a place preference paradigm may not be related to increased novelty seeking in a neophobia paradigm; however they may be associated with attenuated recovery from neophobia, and these two processes may have similar biological mechanisms. NMDA receptors in the amygdala have been shown to be important in both extinction and recovery from neophobia, although they are not necessary for the initial neophobia response (Falls et al. 1992; Lee and Kim 1998; Walker et al. 2002; Botreau et al. 2006; Feltenstein and See 2007; Figueroa-Guzman and Reilly 2008).

In summary, Narp KO mice do not show increased novelty seeking when tested on a neophobia paradigm despite showing increased drug seeking on a morphine CPP paradigm. These mice do, however, show attenuated recovery from neophobia, consistent with previously identified deficits in adapting or shifting behavior to reflect updated values of a reward. As Narp expressed in the prefrontal cortex (Johnson et al. 2010; Blouin et al. 2013) has been shown to mediate these latter phenotypes, future studies should focus on how Narp deletion in this region impacts both recovery from neophobia and updating the value of a reward.

Acknowledgments

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Highlights

We describe a task to assess reactivity to novelty (neophobia) and recovery from neophobia in mice

We find that Narp knockout mice do not have altered reactivity to novelty compared to wild-type mice

Narp knockout mice display behavioral inflexibility and show attenuated recovery from neophobia

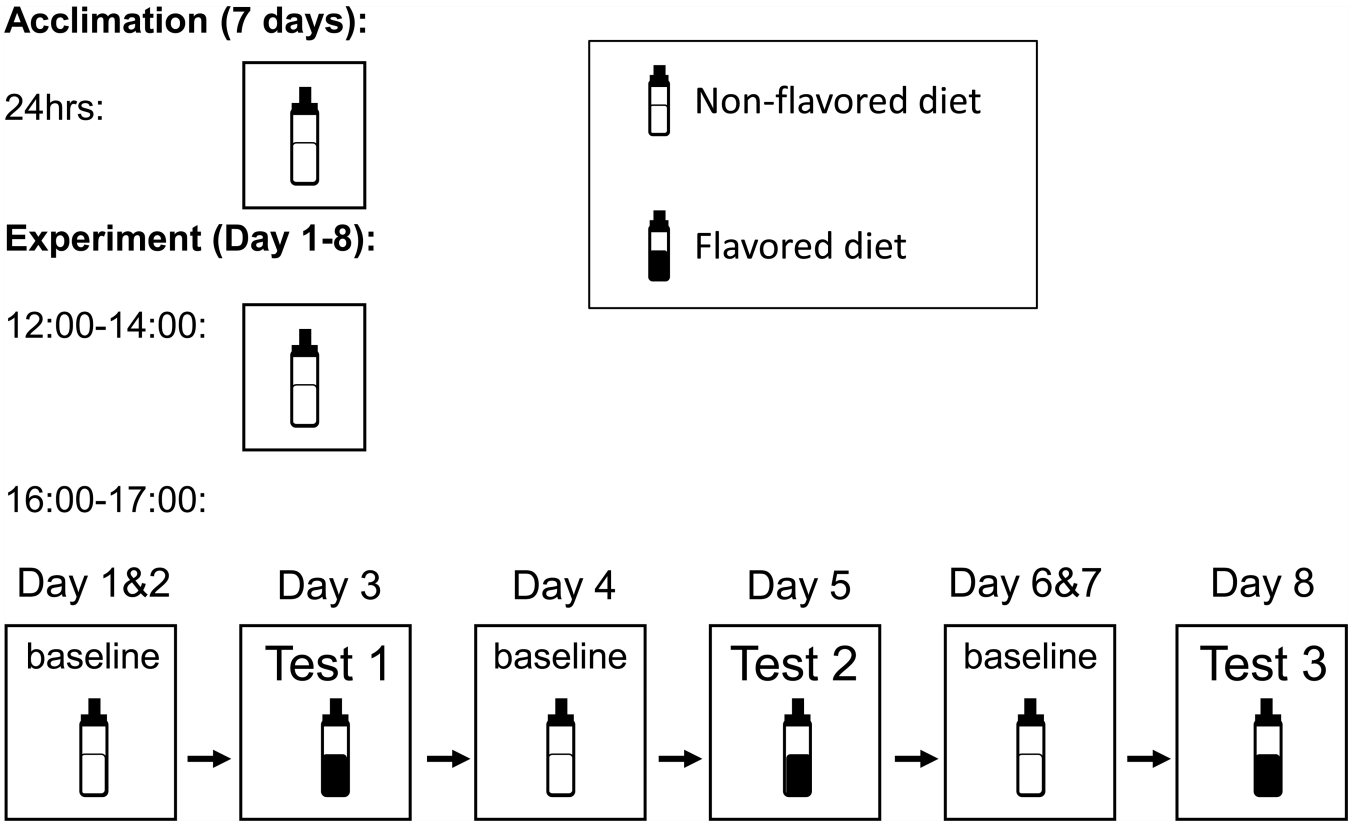

Figure 1.

Neophobia Procedure. During acclimation, mice were given ad libitum access to a rodent liquid non-flavored diet for one week. During the experimental phase, mice were exposed to the liquid diet twice per day for 8 days. On day 1–8, all mice were given a 2 hr exposure (from 12:00–14:00) to the non-flavored diet and a 1 hr exposure (from 16:00–17:00) to either the non-flavored diet or a decaffeinated coffee-flavored diet. Bottles were weighed before and after diet presentations. Consumption is reported in grams of liquid diet consumed or as a suppression ratio. Suppression ratio=grams consumed during Test/(grams consumed during Test + grams consumed during prior baseline day).

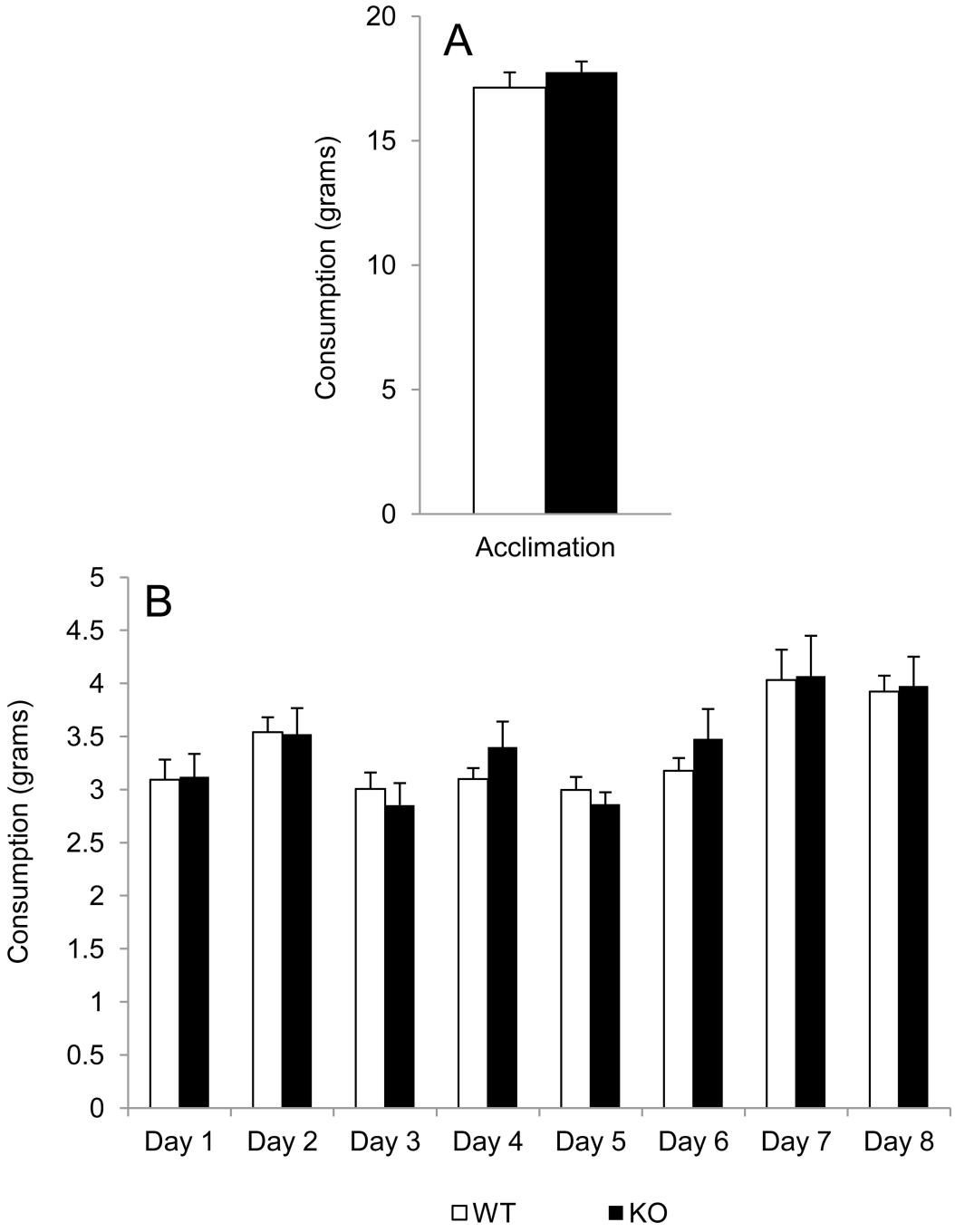

Figure 2.

Level of consumption during acclimation and during the 2 hr exposure to the non-flavored diet. **A**, Narp KO and WT mice drink similar amounts during the acclimation phase (average 24 hr intake) and **B**, during the 2 hr exposures (12:00–14:00) to the non-flavored diet during the experimental phase. Error bars represent ±SEM, n=12 per group.

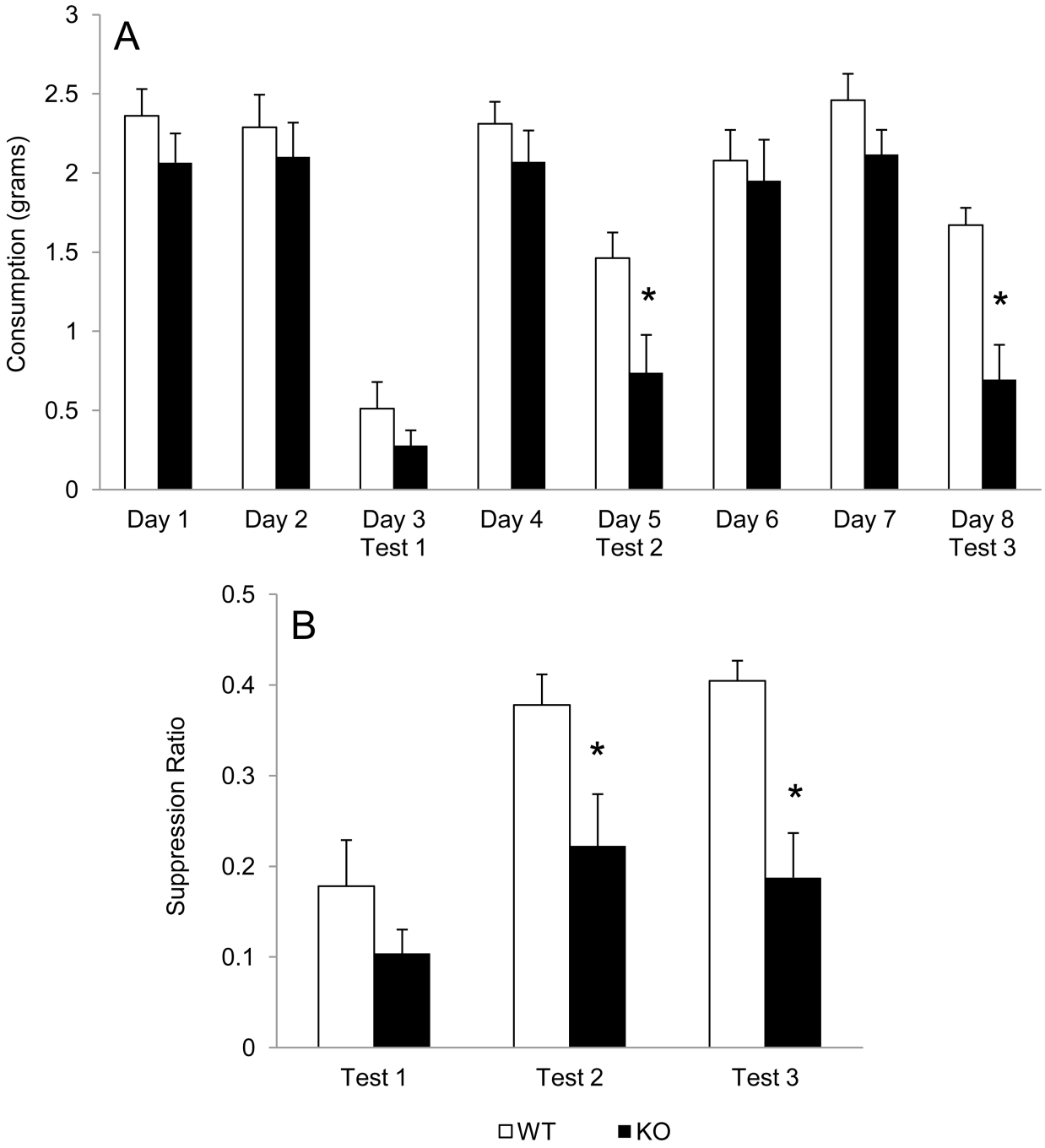

Figure 3.

Narp KO mice show attenuated recovery from neophobia. **A**, Liquid diet consumption during the 1 hr presentation (16:00–17:00) of non-flavored diet (baseline days 1, 2, 4, 6 and 7) or 3% decaffeinated coffee-flavored diet (Test days 3, 5 and 8). Narp KO mice do not differ from WT mice in consumption on baseline days or on Test 1 but show an attenuated increase on Tests 2 and 3. Fisher LSD post-hoc test (*) significant for Test 2 ($p=0.005$) and Test 3 ($p<0.001$). **B**, Test data from 2A presented as a suppression ratio. Fisher LSD post-hoc test (*) significant for Test 2 ($p=0.015$) and Test 3 ($p<0.001$). Suppression ratio=grams

consumed during Test/(grams consumed during Test + grams consumed during prior baseline day). Error bars represent ±SEM, n=11–12 per group.

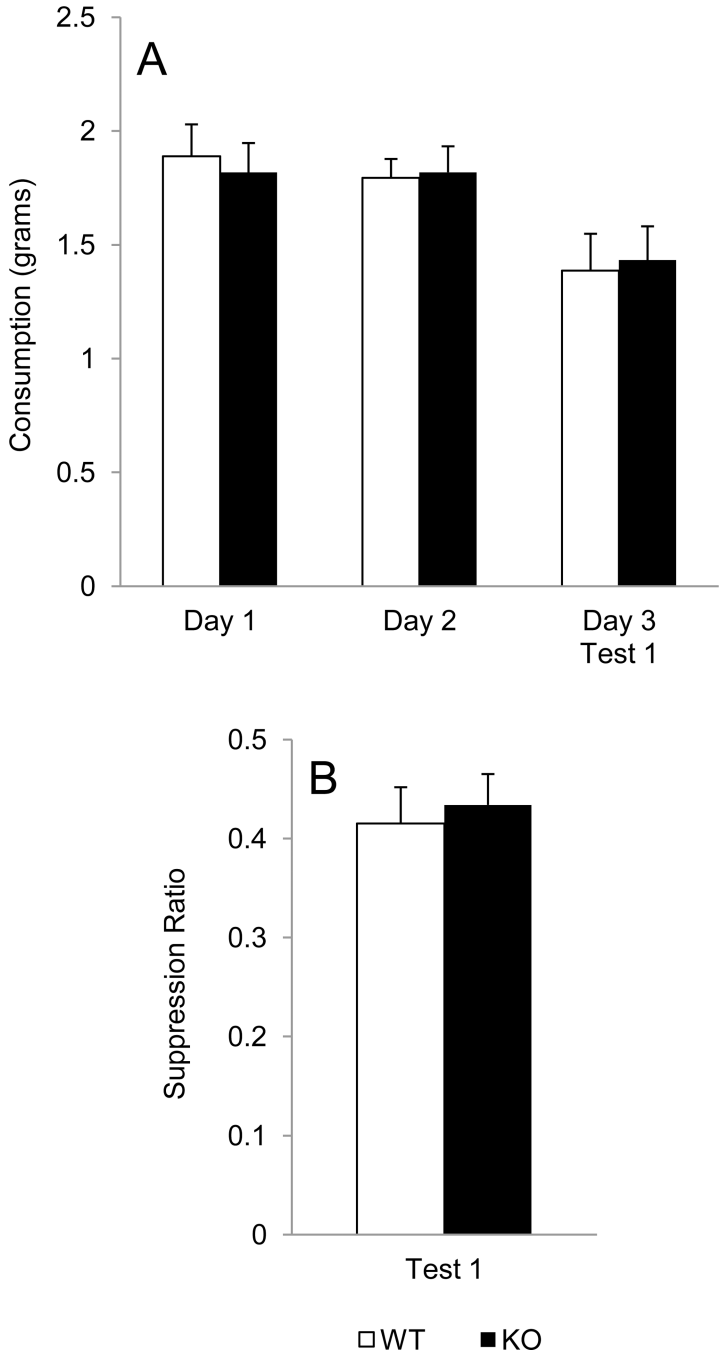

Figure 4.

Narp KO and WT mice show a similar decrease in consumption during the initial presentation of a 1.5% decaf coffee-flavored diet. **A**, Liquid diet consumption during the 1 hr presentation of the non-flavored diet (baseline days 1 and 2) or a 1.5% decaffeinated coffee-flavored diet (Test day 3). Both WT and KOs showed a neophobic response that did not differ with genotype. **B**, Test data from 3A presented as a suppression ratio. Suppression ratio=grams consumed during Test/(grams consumed during Test + grams consumed during prior baseline day). Error bars represent ±SEM, n=15 per group.