REDUCED ADULT NEUROGENESIS SOMETIMES ALTERS BEHAVIOURAL AND ENDOCRINE DISCRIMINATIVE FEAR CONDITIONING. (2013) Jason S Snyder, Heather A Cameron. http://dx.doi.org/10.6084/m9.figshare.884597

The purpose of these experiments was to investigate the role of adult hippocampal neurogenesis in behavioural pattern separation, the ability to perceive, learn and remember fine details of sensory experience. And avoid interference between related experiences. Here, we have used discriminative context fear conditioning to probe this putative function for adult-born neurons. In these paradigms mice or rats are tested in their ability to discriminate (based on their freezing behaviour) a context paired with footshock from a different context that was not paired with footshock.

In the first experiment ("circles vs stripes", Fig 1) we chose a difficult discrimination test, where contexts were very similar except for the pattern on the walls. Wild type mice and neurogenesis-deficient GFAP-TK mice did not "learn" this discrimination. However, WT mice did show discrimination on a test 1 week later, supporting a role for adult neurogenesis in context discrimination/pattern separation and perhaps also in memory consolidation.

In the second experiment ("mo diff", Fig 2) we made the contexts more distinct from each other (more different → "mo diff") to enable WT mice to discriminate during training, and determine whether TK mice showed deficits during the learning phase. They did not (if anything TK mice may have been better discriminators). All mice performed rather poorly in the 1 week tests, however.

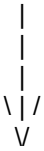
In the third experiment ("stress + mo diff", Fig 3) we repeated the mo diff discrimination but subjected mice to chronic stress prior to training, since chronic stress can enhance fear conditioning (and therefore may enhance 1w memory relative to mo diff) and since neurogenesis-deficient mice have an altered stress response and may learn differently following stress. Here, WT and TK mice both showed strong, similar levels of behavioural discrimination.

Notables:

All WT and TK mice were tested 1 week after training in both contexts in a counterbalanced fashion (i.e. ½ tested in shock context then in safe context, other ½ in safe context then in shock context). The testing order had significant effects on behaviour that were obscured when the tests were pooled. For example, on test 1 in the "circles vs stripes" experiment, WT mice discriminated but TK mice did not. In test 2, WT mice again discriminated but in the "wrong" direction (i.e. they froze more in the safe context, suggesting a carryover effect from test 1; note this effect on test 2 did not reach $p < 0.05$).

In all experiments, in addition to performing behavioural analyses, we also took blood samples after tests 1 and 2 to measure the stress hormone, corticosterone. In some cases corticosterone levels matched behavioural performance, in some cases it didn't. TK mice had much higher levels of corticosterone in the "mo diff' experiment (Fig. 2E).

DATA



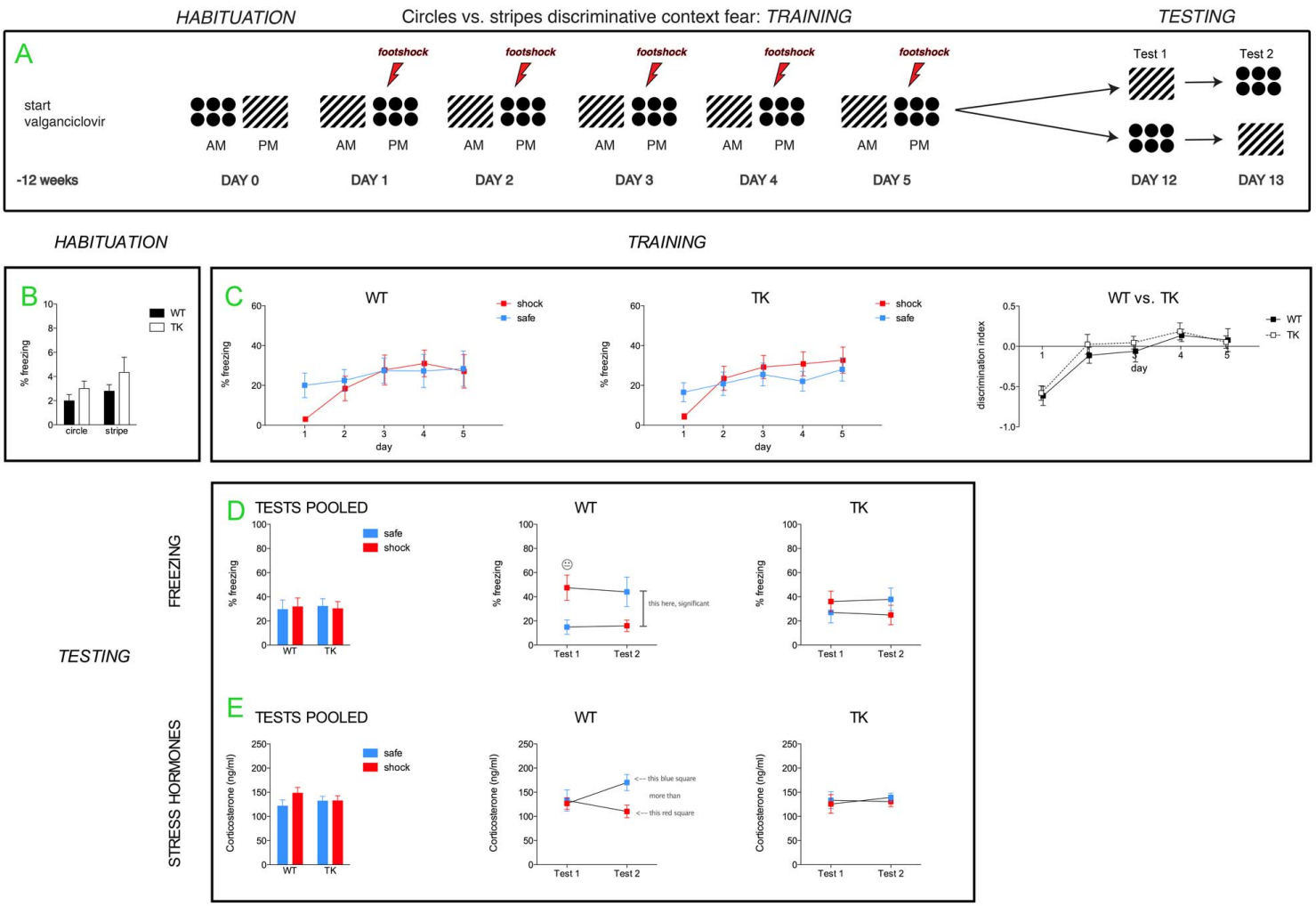


Figure 1: Circles vs Stripes

A) Methods overview: These experiments tested the ability of wild type (N = 12) and neurogenesis-deficient mice (GFAP-TK mice, N = 19) to discriminate 2 contexts in a fear conditioning paradigm. Both contexts were Med Associates fear conditioning chambers that had their 4 walls lined with black and white circle or stripe patterns (mouse always in same chamber, but panels changed). Both patterns were 50% black and 50% white and the 2 contexts did not differ in any other way. Mice were treated for ~12 weeks (beginning at 8 weeks of age) with valganciclovir as described (Snyder, Nature 2011), which completely inhibited adult neurogenesis. Mice were handled daily for 1 week and then given 10 min habituation sessions to each of the 2 contexts (circle in the AM, stripe in the PM). The day following habituation mice were trained for 5 days on the fear conditioning paradigm. Mice were placed in the safe context in the AM for 3 min and in the shock context in the PM for 3 min. In the PM session, mice received a footshock at 2:28 (2s, 0.75mA). Contexts were counterbalanced such that for half of the mice the stripe context was paired with footshock. One week after training half of the mice (i.e. 6 WT, 9 TK) were given a 3min test in the shock context and, the next day, a 3min test in the safe context. The other half of the mice were given a 3min test in the safe context and, the next day, a 3min test in the shock context.

B) Habituation: No significant genotype or context differences in freezing (2 way RM ANOVA)

C) Training: Both WT and TK mice showed an increase in freezing over days but neither genotype showed increased freezing in the shock context (1st 2:28 of each training session shown, i.e. prior to shock). A discrimination index was used to compare genotypes but no significant differences were found. Index = (shock – safe) / (shock + safe); positive values indicate discriminative fear memory.

D) 1 week behaviour tests: When the 1w tests were pooled, there was no context fear discrimination in either genotype (bar graph). However, analyzing by day & context, WT mice froze significantly more when first tested in the shock context (an effect that carried over to the next day's test, when they were tested in the safe context). The unemoticons indicate a significant group effect and significant difference on day 1 (2 way ANOVA with Bonferroni post hoc, both $P < 0.05$). TK mice showed no behavioural discrimination.

E) Corticosterone was measured 30min after the 2 tests (submandibular blood samples). WT mice showed discriminative stress hormone levels on the day 2 test (genotype x context interaction $P = 0.05$, Bonferroni post hoc $P < 0.05$)

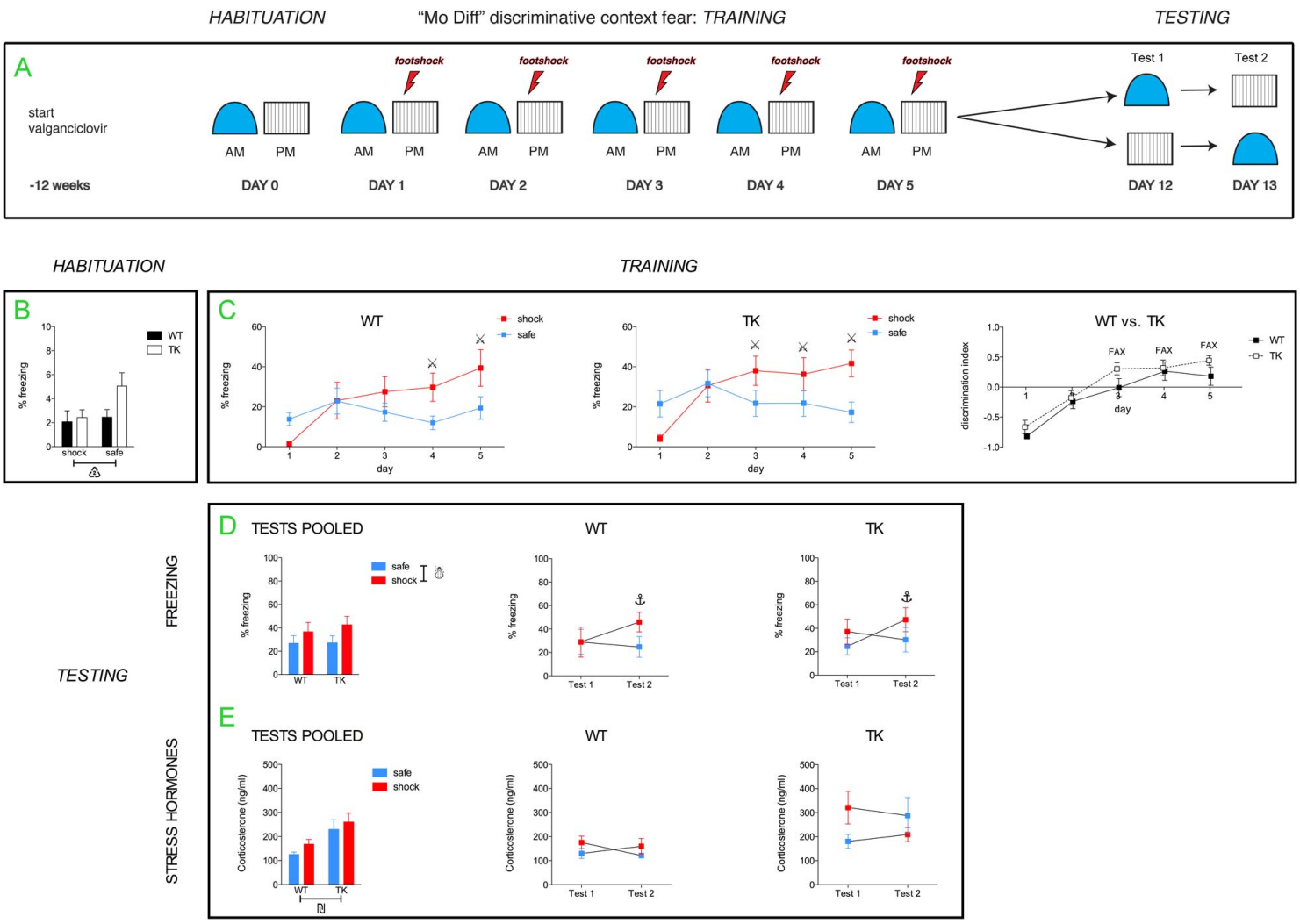


Figure 2: "Mo Diff"

A) Methods Overview: Since mice failed to show behavioural context discrimination during training in the circles vs stripes experiment, contexts were adjusted to make them more different, hence "mo diff". The paradigm is the same as the circles vs stripes experiment except that the shock context was a standard fear conditioning chamber with house and cue lights on, no fan, and was cleaned with dilute Simple Green. The safe context had a solid floor, curved blue walls, houselight only, and was cleaned with vanilla-scented ethanol. For obvious reasons, context-footshock pairing was not counterbalanced. N = 11 WT, 13 TK.

B) Habituation: Mice froze significantly more in the safe context during habituation (context x genotype RM ANOVA, effect of context indicated by the number 2 plastics recycling symbol, $P < 0.05$).

C) Training: WT and TK mice showed increased freezing over days and a significant context x day interaction (2 way RM ANOVA both $P < 0.001$). Mice froze significantly more in the shock context during the latter phases of training, as indicated by the crossed swords (Fisher's LSD, $P < 0.05$). Context discrimination indices were not different between WT and TK mice, but TK mice showed significantly greater discrimination indices than chance on days 3, 4 and 5, as indicated by the word **FAX** (one sample t-test).

D) 1 week behaviour tests revealed significantly greater freezing in the shock context than in the safe context (context x genotype RM ANOVA, effect of context $P < 0.05$, indicated by the snowman). There was a group x day interaction ($P < 0.05$) such that mice that were first tested in the safe context froze significantly more in the shock context on day 2 (Fisher's LSD $P < 0.05$, both WT and TK mice, indicated by the anchors).

E) Pooled 1w corticosterone levels were significantly greater in TK mice than in WT mice (context x genotype RM ANOVA, effect of genotype $P < 0.01$, indicated by the Israeli new Shekel symbol). Context x genotype ANOVAs on each testing day reveal that, in the shock context on day 1 and in the safe context on day 2, TKs had significantly greater corticosterone levels than WT mice (data aren't really graphed to illustrate this).

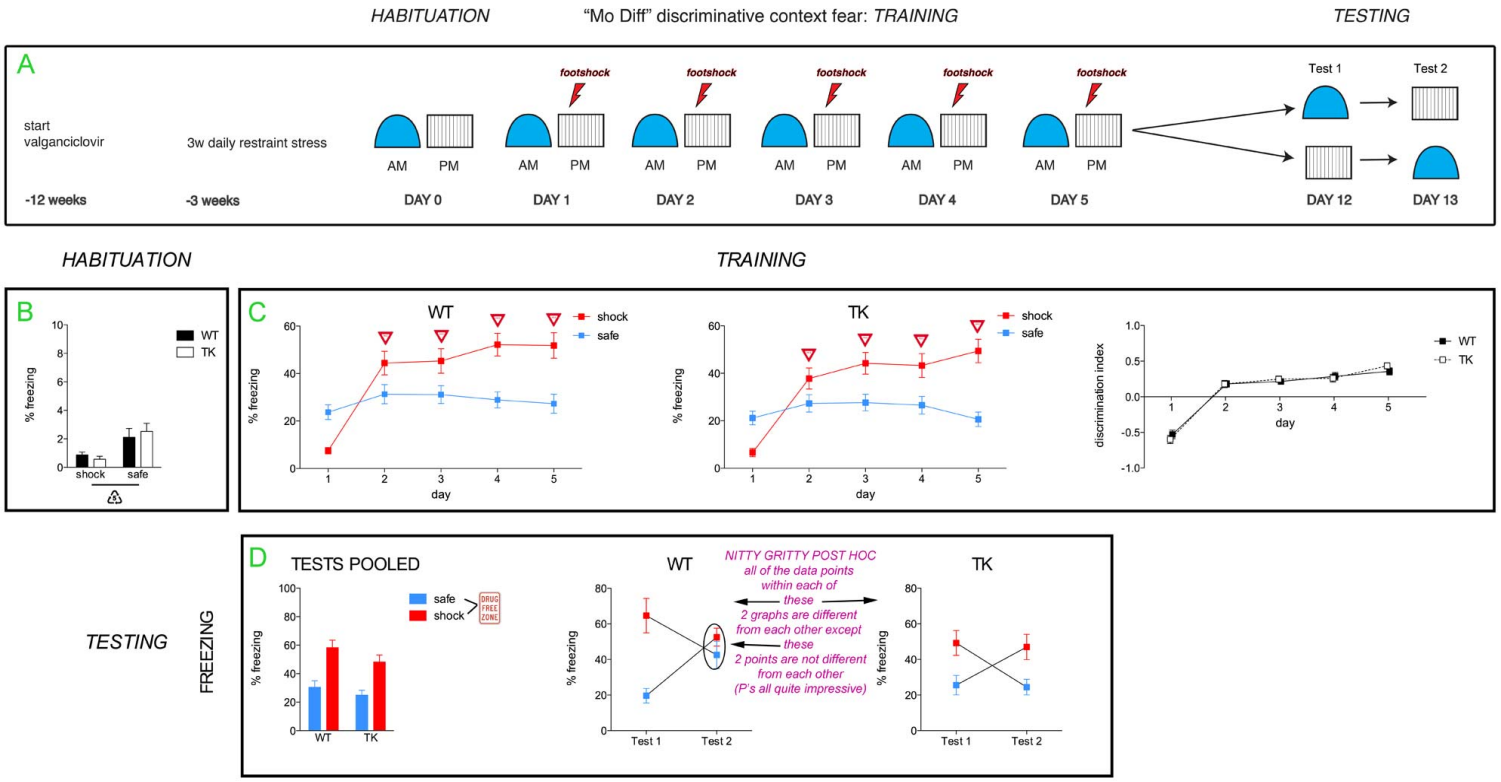


Figure 3: chronic stress + Mo Diff

A) Methods overview: Methods identical to "mo diff" with the exception that mice were subjected to 3 weeks of daily 2hr restraint stress prior to fear conditioning (same mice as in Snyder, Nature 2011 Fig. 2A,B). N = 34 WT, 36 TK.

B) Habituation: Mice froze significantly more in the safe context, in the PM/evening (genotype x context RM ANOVA, main effect of context $P < 0.0001$, indicated by the number 5 plastics recycling symbol).

C) Training: For both WT and TK mice, 2 way RM ANOVAs revealed significant effects of context ($P < 0.05$), day ($P < 0.0001$) and significant interactions ($P < 0.0001$). Both genotypes froze more in the shock context on days 2-5 (Fisher's LSD $P < 0.001$ for all; indicated by yield signs). Comparing freezing discrimination indices, there was no difference between genotypes.

D) 1 week behaviour tests: Pooling the 1w tests, mice froze significantly more in the shock context than in the safe context ($P < 0.001$, indicated by the "drug free zone" sign) and there was no difference between genotypes. Broken down by context testing order, WT mice showed a significant group x day interaction (RM ANOVA, $P < 0.0001$). WT mice successfully discriminated on test day 1 but not day 2 (see figure for post hoc details). TK mice also showed a group x day interaction ($P < 0.0001$), discriminating on *both* day 1 and day 2.

E) Corticosterone levels: Blood samples were obtained 30min after the 1w tests for corticosterone analyses but we never got there. Hence, no panel E.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