Short communication

Resilient rats exhibit better memory performances in spontaneous object-place and object recognition tasks.

Masaharu Ueno, Yukio Ichitani & Kazuo Yamada*

Institute of Psychology and Behavioral Neuroscience, University of
Tsukuba, Tsukuba, Ibaraki 305-8577, Japan

*Correspondence to:

Kazuo Yamada, Ph.D

Institute of Psychology and Behavioral Neuroscience

University of Tsukuba, Tsukuba, Ibaraki 305-8577, Japan

Telefax: +81-29-853-2961

E-mail: kayadama@human.tsukuba.ac.jp

Running title: Resilient rats exhibit better memory

Abstract

Recent studies have investigated individual differences in resilience and vulnerability to stress using fear conditioning and extinction paradigms in rats. In the present study, we investigated whether the individual difference in fear extinction was related to individual's memory function. Thirty-three male Wistar-Imamichi rats were subjected to a spontaneous object-place recognition (SPR) task, an object recognition (SOR) task, and auditory fear conditioning. The rats were assigned to one of three groups: resilient (n=7), vulnerable (n=7) or intermediate (n=9), based on their levels of freezing during fear extinction. Recognition memory performances in the SPR and SOR tasks were compared between groups. Rats in the resilient group were able to discriminate between the novel-place and familiar-place objects in the SPR task, and between the novel and familiar objects in the SOR task, whereas the vulnerable and intermediate groups were not. Rats that exhibited low level of fear in the first trial (n=10) showed novel preference in the SOR task, but not the SPR task. Our results suggest that resilient individuals exhibit better memory and learning ability, and individual differences in resilience and vulnerability to stress may depend on individual's memory function.

Lay summary: The present study showed that rats in the resilient group exhibited rapid extinction of conditioned fear and better performances in the spontaneous object-place and object recognition memory tasks. These findings suggest that individual resilience may depend on learning ability and memory function in rats.

48 **Keyword:** resilience, fear extinction, individual difference, object-place
49 recognition memory, object recognition memory, rats
50

1. Introduction

Exposure to stressful events could elicit subsequent maladaptive symptoms, such as post-traumatic stress disorder. However, only a relatively small population of individuals who have experienced these events develop such pathological responses (Yehuda, Flory, Southwick, & Charney, 2006). Identifying the characteristics that contribute to individual differences could be useful for understanding neurobiological mechanisms of resilience and vulnerability to stress. Previous rodent studies have focused on individual differences in resilience and/or vulnerability (susceptibility) to stress using fear conditioning and extinction paradigms (Bush, Sotres-Bayon, & LeDoux, 2007; King et al., 2018; King, Scott, Graham, & Richardson, 2017; Pajser et al., 2018; Reznikov, Diwan, Nobrega, & Hamani, 2015; Ueno, Yamada, & Ichitani, 2017; Walters, Richardson, & Graham, 2016). Notably, fear expression and extinction could reflect resistance and recovery, both of which are aspects of resilience, respectively (Bush et al., 2007; Pare & Duvarci, 2012; Yehuda et al., 2006).

Fear conditioning and extinction, both of which are indices of resilience, are based on an individual's ability in learning and memory. The learning and memory processes are related to psychobiological resilience (Haglund, Nestadt, Cooper, Southwick, & Charney, 2007). Thus, resilient animals would be able to rapidly recover from detrimental effects of stress and learn about the stressful situation.

Walters et al. (2016) showed that vulnerable rats with high levels of conditioned fear exhibited low expression of a hippocampal neurotrophic factor, as well as a poor performance in a place recognition task. Since the performance in the recognition task depends on the hippocampus (Yamada, Arai, Suenaga, & Ichitani, 2017), hippocampus–

mediated memory function would be related to resilience. To our knowledge, no study has investigated the relationship between resilience based on fear extinction, which is another index of resilience, and memory performance.

Thus, in the present study, we investigated the relationship between the ability to extinguish conditioned fear and memory performance. Given that individual differences in resilience were based on an individual's ability in learning and memory, resilient rats could exhibit high memory performance, whereas vulnerable rats had poor memory.

2. Methods

Thirty-three male Wistar-Imamichi rats (10–13 weeks old) purchased from the Institute for Animal Reproduction (Ibaraki, Japan) were used. Rats were individually housed in a temperature-controlled room (23 ± 1 °C) with a 12:12 light/dark cycle (lights on 8:00 AM) and were allowed ad libitum access to food and water. All behavioral experiments were conducted during the light phase. This study was approved by the University of Tsukuba Committee on Animal Research.

For spontaneous object–place recognition (SPR) and object recognition (SOR) tasks, an open-field arena (90×90×45 cm), with black polyvinyl chloride walls and gray floor (O'Hara & Co., Ltd., Tokyo, Japan), was used. On one of the walls, a white–black vertically striped pattern was attached as an absolute spatial cue. The stimulus objects were copies of 10 different objects made of glass, metal, or plastic and varied in height between 7 and 15 cm. All objects were heavy enough or fixed on the heavy metal plate so that rats could not move them during testing. Different pairs of objects were randomly used in each task and each animal.

107 First, rats underwent 5-min handling for three consecutive days and
108 were habituated to the arena without any objects for 10 min for four
109 consecutive days. The SPR task consisted of a sample phase (10 min)
110 and a test phase (5 min), with a delay interval (10 min) between phases.
111 In the sample phase, two identical objects were placed diagonally in the
112 arena (the center of each object was 22.5 cm from the two adjacent
113 walls), and the rat was released at the center of the arena. Each rat was
114 allowed to freely explore these objects for 10 min. After the sample
115 phase, the rat was removed and returned to its home cage (delay
116 interval). After the delay, the rat was returned to the arena (test phase).
117 One object was placed in the same original position as in the sample
118 phase (familiar object), while the other was moved to a different position
119 (novel object), 30 cm away from the familiar object and 22.5 cm from a
120 sidewall (two possible locations).

121 On the day after the SPR task, the SOR task was conducted, which
122 consisted of a sample phase (5 min) and a test phase (5 min), with a
123 delay interval (5 min) between phases. Procedures in a sample phase and
124 a delay interval were as described in the SPR task. In the test phase, one
125 of the sample objects was replaced by another novel object.

126 In both recognition tasks, the time rats spent exploring the objects
127 was recorded. Exploration was defined as the rat directing its nose
128 toward the object within a distance of 2 cm. In the test phase, the
129 discrimination ratio (DR) was calculated by dividing the amount of
130 exploration of the novel object by the total amount of exploration of the
131 two objects (the familiar and novel objects). The arena was wiped with a
132 wet cloth containing sodium hypochlorite solution, and the objects were
133 cleaned up with 70% ethanol to eliminate odor cues.

Three days after the SOR task, auditory fear conditioning was started based on the previously described procedure (Ueno et al., 2017). For fear conditioning, an automated computer-controlled system (TimeFZ4, O'Hara & Co., Ltd.) was used. In the conditioning phase, each rat was placed into the quadrangular chamber and received seven auditory tone presentations (10 kHz, 65 dB, duration: 30 s) as conditioned stimuli (CS); each tone was delivered concomitant with a foot-shock (0.7 mA, duration: 1 s) as unconditioned stimuli. In the extinction trials, each rat was placed into the triangular chamber and exposed to five tone presentations without foot-shocks, for three consecutive days. Freezing (conditioned fear responses) was defined as the absence of all movements lasting at least 5 s, except for those associated with breathing, and scored only during tone presentations.

According to previous studies (Bush et al., 2007; King et al., 2018; King et al., 2017), rats that displayed <50% freezing in the first extinction trial were excluded for the analysis of fear extinction. The exploration time in recognition tasks and the freezing level (%) during fear conditioning were analyzed using two-way analysis of variance (ANOVA) or repeated measures ANOVA. Individual comparisons were evaluated using a simple main effect test and a post-hoc Bonferroni test. In recognition tasks, DRs were compared between groups using one-way ANOVA, and to theoretical chance level (50%) using a one-sample *t*-test (Gaskin et al., 2010). In the analysis of DRs, we included the rats that were excluded due to the failure of fear conditioning.

3. Results

We excluded ten rats from the analysis of fear extinction because they did not reach the criterion in fear learning. The levels of freezing in

three extinction trials in all rats ($n=23$) are shown in Fig. 1 (a). A significant main effect was observed in the trials ($F(2, 44) = 23.70$, $p < .01$, $\eta^2 = .22$), and the post-hoc comparison revealed that the freezing level in the third trial was significantly lower than that in the first and second trials ($p < .05$), and the freezing level in the second trial was significantly lower than that in the first trial ($p < .05$).

Based on the freezing level in the third extinction trial, as previously described (Ueno et al., 2017), we assigned the rats to three groups: resilient, vulnerable, and intermediate. Rats that exhibited freezing in the lower 30% of the distribution were regarded as the resilient group ($n=7$), while those in the higher 30% were regarded as the vulnerable group ($n=7$). The remaining 40% were assigned as the intermediate group ($n=9$). The levels of freezing in the extinction trials for each group are shown in Fig. 1 (b). Significant main effects were observed in the groups ($F(2, 20) = 21.86$, $p < .01$, $\eta^2 = .69$) and trials ($F(2, 40) = 26.89$, $p < .01$, $\eta^2 = .57$), and a significant interaction was also observed between them ($F(4, 40) = 3.28$, $p < .05$, $\eta^2 = .25$). A post-hoc comparison revealed that the freezing levels in the resilient group were lower than those in the vulnerable group in all trials ($p < .05$). Rats in the intermediate group showed higher levels of freezing than those in the resilient group in the first and third trials ($p < .05$) and lower levels of freezing than those in the vulnerable group in the third trial ($p < .05$). In addition, the freezing level in the third trial was lower than that in the first and second trials in the resilient and intermediate groups ($p < .05$). However, there was no significant difference in the freezing levels among trials in the vulnerable group.

The levels of freezing during five CS presentations in the first extinction trial are shown in Fig. 1 (c). Significant main effects were

observed in the groups ($F(2, 20) = 7.78, p < .01, \eta_p^2 = .44$) and trials ($F(4, 80) = 6.35, p < .01, \eta_p^2 = .24$), but no significant interaction was observed between them ($F(8, 80) = 1.64, \eta_p^2 = .14$). The post-hoc comparison revealed that the resilient group exhibited lower levels of freezing than the intermediate and vulnerable groups ($p < .05$), and the freezing level in the second CS was higher than those in the fourth and fifth CS ($p < .05$).

In the analysis of recognition memory performances, rats that were excluded for the analysis of fear extinction were included as a low fear group ($n=10$). The exploration time for each object in the sample phase of the SPR task was not significantly different among groups (Low fear: 72.22 ± 10.63 s, 63.95 ± 9.22 s; Resilient: 57.27 ± 6.91 s, 42.38 ± 4.70 s; Intermediate: 56.82 ± 10.86 s, 56.55 ± 7.13 s; Vulnerable: 72.19 ± 11.74 s, 52.25 ± 12.47 s). In the sample phase of the SOR task, there was no significant difference in the exploration time for each object (Low fear: 37.05 ± 6.70 s, 31.83 ± 4.79 s; Resilient: 44.58 ± 4.58 s, 36.04 ± 3.15 s; Intermediate: 37.95 ± 6.78 s, 43.70 ± 6.12 s; Vulnerable: 42.33 ± 5.92 s, 29.65 ± 9.22 s). Figure 2 (a) shows DRs in the SPR and SOR tasks for all rats ($n=33$). The DRs in both SPR and SOR tasks were significantly above chance levels (SPR: $t(32) = 2.37, p < .05, r = .39$, SOR: $t(32) = 3.50, p < .01, r = .53$).

Figure 2 (b) shows the DRs in the SPR task for each group. Only the DR in the resilient group was significantly higher than the chance level ($t(6) = 2.86, p < .05, r = .76$). No significant differences between groups were observed ($F(3, 29) = 1.35, \eta^2 = .12$). Figure 2 (C) shows the DRs in the SOR task for each group. The DRs in the resilient and low fear groups were significantly higher than the chance level (resilient: $t(6) = 5.22, p < .01, r = .91$; low fear: $t(9) = 2.57, p < .05, r = .65$). No significant

differences between groups were observed ($F(3, 29) = 0.70$, $\eta^2 = .07$).

4. Discussion

To investigate whether the individual difference in fear extinction was related to recognition memory function, rats were assigned to three groups (resilient, vulnerable, and intermediate) based on their performance in fear extinction and then their performance in the SPR and SOR tasks were compared. Rats in the resilient group exhibited rapid extinction within the first trial and low levels of freezing throughout the extinction trials, whereas rats in the vulnerable group maintained the high level of freezing, showing a clear individual difference in fear extinction. Our results were consistent with those of the previous studies that found the individual difference in fear extinction (Bush et al., 2007; King et al., 2017; Reznikov et al., 2015).

In both SPR and SOR tasks, the DRs were significantly higher than chance level in the analysis for all animals. However, in the analysis of each group, the DRs were significantly higher than chance level in the resilient, but not in the vulnerable and intermediate groups. Because there were no differences in the total exploration time in the sample phase of the SPR and SOR tasks among the groups, the possibility that sensorimotor or motivational aspects of performance were different among the groups can be ruled out. Therefore, resilient rats have a better ability to perform both of the SPR and SOR tasks.

In addition, rats in the low fear group, which failed to develop conditioned fear responses, also exhibited better performance only in the SOR task. In contrast, Walters et al. (2016) found that rats with low fear expression exhibited intact performances in the SPR and SOR tasks. Only rats with high fear expression exhibited poor performances in the

246 SPR task. The discrepancy with Walters' study may be due to the
247 differences not only in strains used but also in the procedures of fear
248 conditioning. While severe foot-shocks (0.7 mA, 1 s $\times$ 7) were given in
249 this study, Walters et al. (2016) used only one shock (0.6 mA, 1 s) for
250 fear conditioning. The obvious difference in the procedures of fear
251 conditioning could cause the difference in the strength of conditioned
252 fear. Walters et al. (2016) assigned rats to three groups based on fear
253 expression levels, whereas our classification focused on individual
254 performance in fear extinction, which reflected a different aspect of
255 resilience and neurobiological mechanisms (Bush et al., 2007; Holmes &
256 Singewald, 2013; Pare & Duvarci, 2012; Yehuda et al., 2006).

257 The fact that rats in the low fear group could not develop
258 conditioned fear responses even with the exposure to severe shocks
259 could be interpreted as that they have high stress resistance or low
260 associative learning ability. Thus, our results that rats in the low fear
261 group could exhibit novel preference in the SOR task, but not the SPR
262 task, may be based on differences in the difficulty of each task,
263 especially the duration in sample phases. In the SOR task, rats could
264 discriminate between the novel and familiar objects by the investigation
265 of the sample object for 90 s during familiarization (Gaskin et al., 2010),
266 while in the SPR task, the 20-min sample phase is necessary to exhibit
267 novel preference (Ozawa, Yamada, & Ichitani, 2011). Thus, the low fear
268 group could perform better in the easier task but not in the more difficult
269 task.

270 The SPR and SOR tasks are involved with different brain regions— the
271 hippocampus and the perirhinal cortex, respectively. Rats in the resilient
272 group exhibited better performances in both tasks, suggesting that the
273 ability to recover from stress may be related to the function of those brain

regions. In particular, the regulation of N-methyl-D-aspartate receptors in the hippocampus could be related to both of the spatial learning ability and stress resilience (Adams et al., 2001; Haglund et al., 2007; Horn, Charney, & Feder, 2016).

Therefore, resilient rats exhibited better performances in both SPR and SOR tasks, suggesting that the individual's ability to rapidly recover from stressful situations may be based on their learning and memory functions.

Disclosure statement

No conflict of interest was reported by the authors.

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References

- Adams, M. M., Smith, T. D., Moga, D., Gallagher, M., Wang, Y., Wolfe, B. B., ... Morrison, J. H. (2001). Hippocampal dependent learning ability correlates with N-methyl-D-aspartate (NMDA) receptor levels in CA3 neurons of young and aged rats. *Journal of Comparative Neurology*, 432(2), 230–243. <https://doi.org/10.1002/cne.1099>
- Bush, D. E. A., Sotres-Bayon, F., & LeDoux, J. E. (2007). Individual differences in fear: Isolating fear reactivity and fear recovery phenotypes. *Journal of Traumatic Stress*, 20(4), 413–422. <https://doi.org/10.1002/jts.20261>
- Gaskin, S., Tardif, M., Cole, E., Piterkin, P., Kayello, L., & Mumby, D. G. (2010). Object familiarization and novel-object preference in rats. *Behavioural Processes*, 83(1), 61–71. <https://doi.org/10.1016/j.beproc.2009.10.003>
- Haglund, M. E. M., Nestadt, P. S., Cooper, N. S., Southwick, S. M., & Charney, D. S. (2007). Psychobiological mechanisms of resilience: Relevance to prevention and treatment of stress-related psychopathology. *Development and Psychopathology*, 19(3), 889–920. <https://doi.org/10.1017/S0954579407000430>
- Holmes, A., & Singewald, N. (2013). Individual differences in recovery from traumatic fear. *Trends in Neurosciences*, 36(1), 23–31. <https://doi.org/10.1016/j.tins.2012.11.003>
- Horn, S. R., Charney, D. S., & Feder, A. (2016). Understanding resilience: New approaches for preventing and treating PTSD. *Experimental Neurology*, 284, 119–132. <https://doi.org/10.1016/j.expneurol.2016.07.002>
- King, G., Graham, B. M., & Richardson, R. (2018). Individual differences in fear relapse. *Behaviour Research and Therapy*, 100(November 2017), 37–43. <https://doi.org/10.1016/j.brat.2017.11.003>
- King, G., Scott, E., Graham, B. M., & Richardson, R. (2017). Individual differences in fear extinction and anxiety-like behavior. *Learning*

326 *and Memory*, 24(5), 182–190.
327 <https://doi.org/10.1101/lm.045021.117>

328 Ozawa, T., Yamada, K., & Ichitani, Y. (2011). Long-term object location
329 memory in rats: Effects of sample phase and delay length in
330 spontaneous place recognition test. *Neuroscience Letters*, 497(1),
331 37–41. <https://doi.org/10.1016/j.neulet.2011.04.022>

332 Ozawa, T., Yamada, K., & Ichitani, Y. (2014). Hippocampal BDNF
333 treatment facilitates consolidation of spatial memory in
334 spontaneous place recognition in rats. *Behavioural Brain Research*,
335 263, 210–216. <https://doi.org/10.1016/j.bbr.2014.01.034>

336 Pajser, A., Breen, M., Fisher, H., & Pickens, C. L. (2018). Individual
337 differences in conditioned fear are associated with levels of
338 adolescent/early adult alcohol consumption and instrumental
339 extinction. *Behavioural Brain Research*, 349(December 2017), 145–
340 157. <https://doi.org/10.1016/j.bbr.2018.04.020>

341 Pare, D., & Duvarci, S. (2012). Amygdala microcircuits mediating fear
342 expression and extinction. *Current Opinion in Neurobiology*, 22(4),
343 717–723. <https://doi.org/10.1016/j.conb.2012.02.014>

344 Reznikov, R., Diwan, M., Nobrega, J. N., & Hamani, C. (2015). Towards a
345 better preclinical model of PTSD: Characterizing animals with weak
346 extinction, maladaptive stress responses and low plasma
347 corticosterone. *Journal of Psychiatric Research*, 61, 158–165.
348 <https://doi.org/10.1016/j.jpsychires.2014.12.017>

349 Ueno, M., Yamada, K., & Ichitani, Y. (2017). The relationship between
350 fear extinction and resilience to drug-dependence in rats.
351 *Neuroscience Research*, 121, 37–42.
352 <https://doi.org/10.1016/j.neures.2017.03.006>

353 Walters, E., Richardson, R., & Graham, B. M. (2016). Individual
354 differences in conditioned fear expression are associated with
355 enduring differences in endogenous Fibroblast Growth Factor-2 and
356 hippocampal-mediated memory performance. *Neurobiology of*
357 *Learning and Memory*, 134(Part B), 248–255.
358 <https://doi.org/10.1016/j.nlm.2016.07.021>

- 359 Yamada, K., Arai, M., Suenaga, T., & Ichitani, Y. (2017). Involvement of
360 hippocampal NMDA receptors in encoding and consolidation, but
361 not retrieval, processes of spontaneous object location memory in
362 rats. *Behavioural Brain Research*, 331(April), 14–19.
363 <https://doi.org/10.1016/j.bbr.2017.05.006>
- 364 Yehuda, R., Flory, J. D., Southwick, S., & Charney, D. S. (2006).
365 Developing an agenda for translational studies of resilience and
366 vulnerability following trauma exposure. *Annals of the New York*
367 *Academy of Sciences*, 1071, 379–396.
368 <https://doi.org/10.1196/annals.1364.028>

Figure legends

Fig. 1. (a) Mean (SEM) percentage of freezing during extinction trials for all rats (n=23) and (b) each group (resilient (n=7), vulnerable (n=7), and intermediate (n=9)). (c) Mean (SEM) percentage of freezing during the first extinction trial for each group. $*p < .05$.

Fig. 2. (a) Mean (+SEM) discrimination ratios (DRs) in the test phase of SPR and SOR tasks for all rats (n=33). Mean (+SEM) DRs in the test phase of SPR (b) and SOR (c) for each group (low fear (n=10), resilient (n=7), vulnerable (n=7), and intermediate (n=9)). Rats that were excluded for the analysis of fear extinction were included as low fear group. The analysis was conducted on the first 2 min of the test phase to reliably measure rats' novel preference (Ozawa, Yamada, & Ichitani, 2014). $*p < .05$, $**p < .01$ compared to chance level (50%).

Fig. 1

Fig. 2