

## RESEARCH HIGHLIGHT

# 5-hydroxytryptamine type-3A receptor in the process of fear extinction

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Received: January 23, 2014

Published online: February 18, 2014

**The 5-hydroxytryptamine type-3 (5-HT<sub>3</sub>) receptor is the only ligand-gated ion channel in the 5-HT receptor subfamilies. Although it is known that the 5-HT<sub>3</sub> receptor is expressed in the hippocampus, amygdala and prefrontal cortex, it is not well studied whether it has roles in fear memory processes. We performed behavioral analyses of 5-HT<sub>3A</sub> receptor knockout mice in fear conditioning tests and found that the 5-HT<sub>3</sub> receptor is indispensable for the fear extinction, but not for the acquisition or retention of fear memory. Furthermore, we found the 5-HT<sub>3A</sub> receptor contributes to the specificity of context in fear extinction processes. Our results indicate the 5-HT<sub>3A</sub> receptor is one of the key molecules for regulation of fear extinction. Moreover, it could be an important treatment target for the fear-related disorders, such as post-traumatic stress-disorder (PTSD).**

**To cite this article:** Kondo M, et al. 5-hydroxytryptamine type-3A receptor in the process of fear extinction. *Receptor Clin Invest* 2014; 1: e71. doi: 10.14800/rci.71.

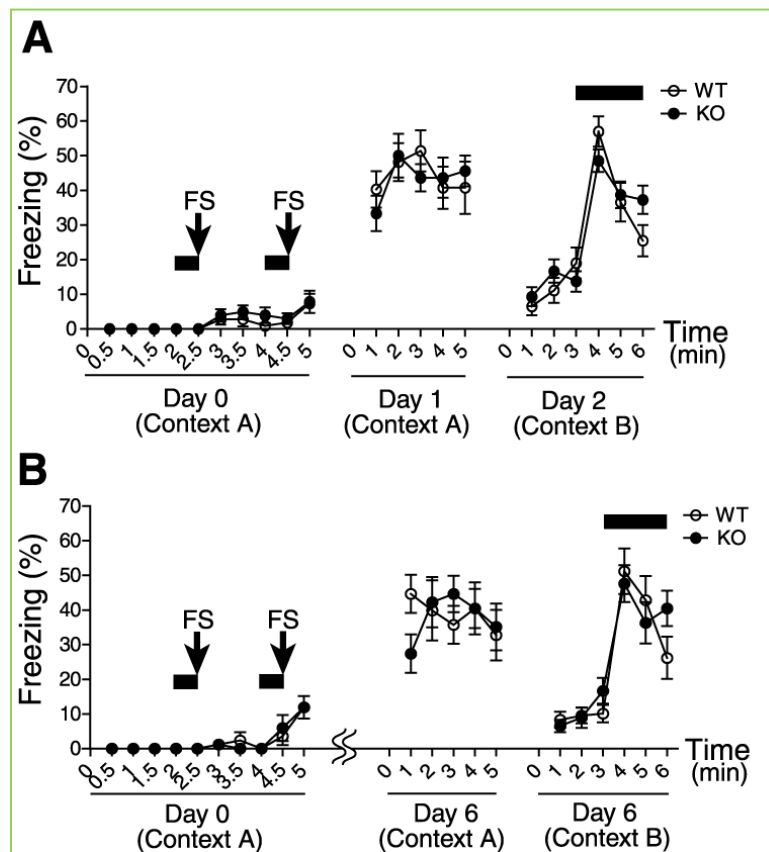
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Fear is a central emotion for organizing defensive action under threat; therefore, it is quite important for animals to survive. Dysfunction of fear system gives rise to inappropriate fears that lead to fear-related disorders, such as post-traumatic stress-disorder (PTSD) [1,2,3]. In general, the treatment of these diseases involves the modulation of fear memory [4]. To develop the effective treatment of fear-related disorders, it is quite fundamental to understand the mechanisms of the processes of fear memory.

The 5-hydroxytryptamine type-3 (5-HT<sub>3</sub>) receptor is the only ligand-gated ion channel in the 5-HT receptor subfamilies [5]. The subunit of 5-HT<sub>3A</sub> is indispensable subunit to function as a 5-HT<sub>3</sub> receptor. [6,7]. Mainly, the 5-HT<sub>3A</sub> receptor is expressed in neurons of brain regions including hippocampus and amygdala [8,9,10], indicating the

participation in higher brain functions, such as cognition and emotion. Although it has been reported that the 5-HT<sub>3</sub> receptor has roles in spatial memory [11,12], anxiety [13,14] and social behavior [15], it is not well studied whether the 5-HT<sub>3</sub> receptor plays possible roles in the process of fear memory. In our recent study entitled “the 5-HT<sub>3A</sub> receptor is essential for fear extinction [16]” we used 5-HT<sub>3A</sub> receptor knockout (*5-ht3ar*<sup>-/-</sup>) mice and performed a fear conditioning test and fear extinction trial.

The fear conditioning test is one of the behavioral tests which assess associative learning between an aversive foot shock (unconditioned stimulus) and a conditioned stimulus [16,17]. On the conditioning day (Day 0), the mouse was put in the conditioning chamber (Context A) for 120 sec and presented with a tone (CS: 85 dB, 3000 Hz) for 30



**Figure 1. The 5-HT3A receptor is not essential for the acquisition or retention of fear memory.** Neither acquisition (A) nor retention (B) of fear memory was affected in *5-ht3ar*<sup>-/-</sup> mice. Mean percentage of freezing time averaged every minute during the fear conditioning test. Bars represent the tone presentation. Arrows represent the foot shock. FS, foot shock. (A, WT, n = 18; KO, n = 17 mice; B, both groups, n = 14 mice). Means ± SEM are shown in all histograms.

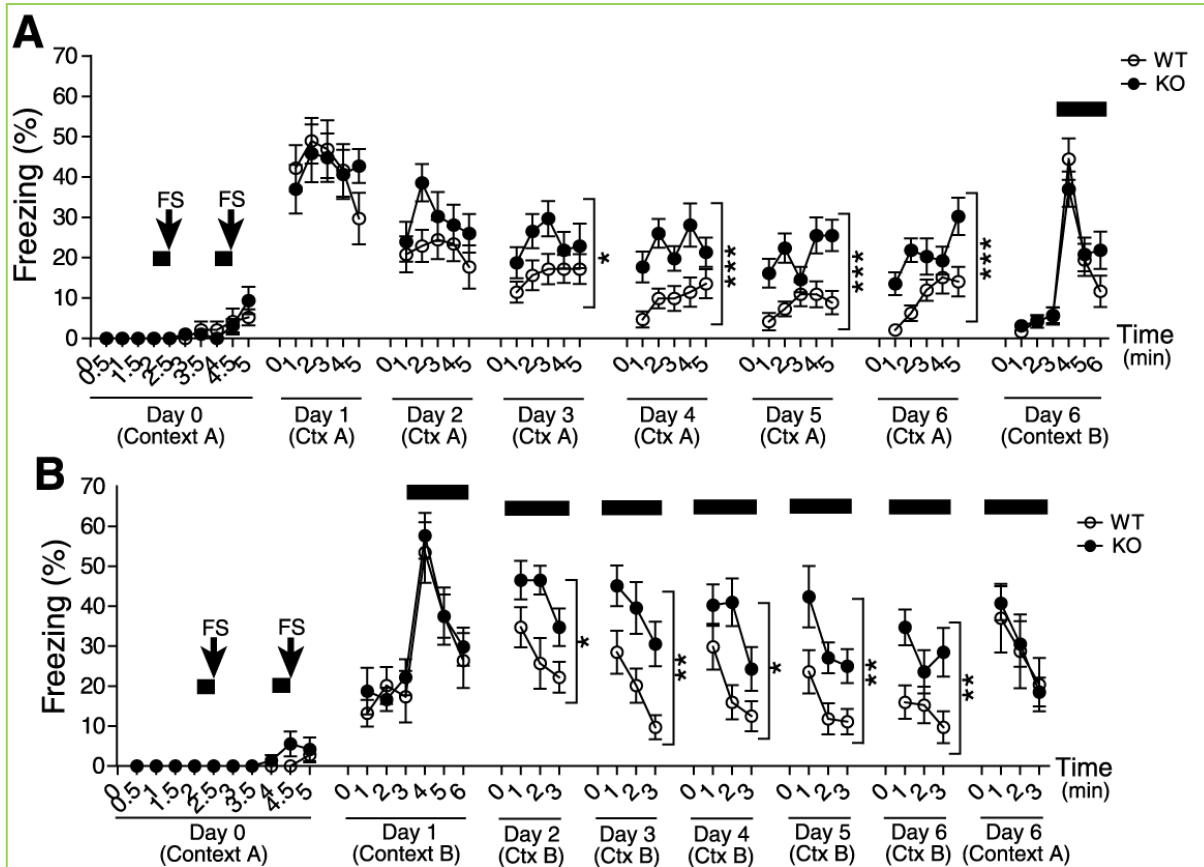
sec. At the end of the tone, the mouse was given a foot shock (US: 2 sec, 0.6 mA). One more tone-shock pairing was presented with a 120 sec inter-stimulus interval. Thirty seconds after the final foot shock, the mouse was returned to the home cage. For the contextual fear test, the mouse was put in the conditioning chamber (Context A), whereas for the tone-cued fear test, the mouse was put in a novel chamber (Context B) and exposed to tone presentation.

As the abnormalities in pain sensitivity or motor activity have inappropriate effects on the fear conditioning tests, we first confirmed that *5-ht3ar*<sup>-/-</sup> mice exhibit no malformation of pain sensitivity or no dysfunction of motor activity.

We tested whether the 5-HT3A receptor has roles in the acquisition and retention of fear memory in the fear conditioning test. After the conditioning (Day 0), the contextual fear test was performed on Day 1 and the tone-cued fear test was done on Day 2. No significant differences were observed in contextual freezing behaviors in the context A, and in the tone-cued freezing

behaviors in the context B between wild-type and *5-ht3ar*<sup>-/-</sup> mice (Figure 1A). Next, we tested the retention of fear memory in the contextual and tone-cued fear tests on Day 6. Both the contextual freezing behaviors in the context A and in the tone-cued freezing behaviors in the context B were similar between wild-type and *5-ht3ar*<sup>-/-</sup> mice (Figure 1B). Our results indicate the 5-HT3A receptor is not essential for the acquisition or retention of fear memory.

Then, we tested whether the 5-HT3A receptor has possible roles in the fear extinction processes. The fear conditioning was performed in the conditioning chamber (Context A) on Day 0. Thereafter, the mouse was subjected to the daily extinction trial. We recorded freezing behaviors on each day (Day 1–Day 6). The first extinction trial assesses the contextual fear extinction. The mouse was re-exposed to the conditioned chamber (context A) for five minutes without the foot shocks. In contrast to wild-type mice, a significant impaired decrease in freezing responses was seen in the *5-ht3ar*<sup>-/-</sup> mice, suggesting the contextual fear extinction was impaired



**Figure 2. The 5-HT3A receptor is indispensable for the extinction of fear memory.** The extinction of both contextual (A) and tone-cued (B) fear were impaired in *5-ht3ar*<sup>-/-</sup> mice. Mean percentage of freezing time averaged every minute during the fear extinction trials. Bars represent the tone presentation. Arrows represent the foot shock. FS, foot shock. \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001 (two-way repeated-measures ANOVA). (A, n = 16 mice; B, n = 12 mice). Means ± SEM are shown in all histograms.

(Figure 2A). In the post-extinction tests performed on Day 6, there were no significant differences in freezing behaviors in a novel context (context B) between wild-type and *5-ht3ar*<sup>-/-</sup> mice, indicating that the different freezing responses in the extinction processes between wild-type and *5-ht3ar*<sup>-/-</sup> mice were specific to the extinction context (context A). Our results suggest that the 5-HT3A receptor is indispensable for the contextual fear extinction.

The second extinction trial assesses the tone-cued fear extinction. The mouse was exposed to the tone in a novel chamber (context B) for three minutes without the foot shocks. We recorded the tone-cued freezing behaviors on each day (Day 1 - Day 6). In contrast to wild-type mice, a significant impaired decrease in freezing responses was observed in *5-ht3ar*<sup>-/-</sup> mice, suggesting the tone-cued fear extinction was also impaired (Figure 2B).

In the post extinction tests performed on Day 6, there were no significant differences in tone-cued freezing behaviors in the context A between wild-type and *5-ht3ar*<sup>-/-</sup> mice, indicating the different freezing responses in the

fear extinction processes were specific to the extinction context. Our results indicate that the 5-HT3A receptor is indispensable for the tone-cued fear extinction.

The renewal effect is one of the characteristics of context-specific extinction [18,19,20]. It is known that the conditional fear behaviors re-emerges after the conditioned stimulus is exhibited in a different context from the context in the extinction trials. In our results, surely, in wild-type mice, significant higher freezing behaviors were observed in context A than context B. This is the renewal effects. On the other hand, intriguingly, *5-ht3ar*<sup>-/-</sup> mice did not show such renewal effect (Figure 2B). Our results support the idea that the 5-HT3A receptor is involved in the specificity of context in fear extinction processes.

In the study [16], we found that the 5-HT3A receptor is indispensable for the fear extinction, but not for the acquisition or retention of fear memory.

The distributed and complex neuronal circuit, including the hippocampus, amygdala and prefrontal cortex, is

known to mediate the processes of fear extinction [2,3,19,20]. The 5-HT3A receptor is known to be expressed in GABAergic neurons in these limbic regions [10,21,22]. Moreover, GABAergic neurons are excited directly through the activation of 5-HT3A receptors [23,24,25] and the 5-HT3A receptor regulates GABA neurotransmission [26,27,28,29]. Furthermore, it has been reported that GABA neurotransmitter system is involved in fear extinction processes through the modulation of neuronal network [2,30,31]. Taken all together, those reports and our findings raised the possibility that the 5-HT3A receptor contributes to the fear extinction processes via GABA neurotransmitter systems. It is reported that interneurons which express the 5-HT3 receptor comprise heterogeneous subpopulations, which are involved in different neuronal circuits [10,22,32]. However, the specific functions of respective neuronal subpopulations in the processes of fear memory are not well studied. Further analyses which are based on the subpopulations of the interneurons expressing the 5-HT3 receptor could give us depthful insight into the respective specific functions and roles in the processes of fear memory.

The complex neuronal circuit associated with a lot of brain regions is implicated in the extinction processes [2,3,19,20]. At the present time, however, it is not known what roles of the 5-HT3A receptor are in these brain regions in extinction processes of fear memory. Moreover, it is not known which brain regions are critically involved in the fear extinction processes mediated by the 5-HT3A receptor, as well. Further and detailed studies are necessary to solve these questions.

It is known that PTSD is highly related to impairment in extinction of fear memory [4,33,34]. Although previous reports have indicated that serotonin neurotransmitter is associated with PTSD, how serotonin acts in the pathophysiology of PTSD is not well understood. Our recent report [16] is the first report to show directly that the 5-HT3A receptor contributes specifically to the extinction processes of fear memory by behavioral analyses in 5-HT3A receptor knockout mice. The 5-HT3A receptor is one of the key molecules for the regulation of fear extinction. Moreover, it could be a critical treatment target for fear-related disorders, such as PTSD.

### Conflicting interests

The authors have declared that no competing interests exist.

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