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Viral vector-mediated blockade of the endocrine stress-response modulates non-spatial memory

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Abstract

Stress results in the release of glucocorticoids (GCs) which, at high levels, impair hippocampus-dependent tasks. Estrogen is neurotrophic and can rescue stress-induced memory impairments. Here we report the use of a viral-vector to overexpress a chimeric gene (ER/GR) that converts the deleterious effects of glucocorticoids into beneficial estrogenic effects. A short immobilization stress regimen was sufficient to impair non-spatial memory. In contrast, viral vector-mediated overexpression of ER/GR in the dentate gyrus of the hippocampus protected against stress-induced impairments of non-spatial memory. These data add to the growing evidence that increasing estrogenic signaling can protect against the impairing effects of stress on non-spatial memory.

Hippocampal memory is sensitive to glucocorticoids (GCs, the adrenal steroids secreted during stress) in an inverted-U manner, such that low to moderate GC concentrations enhance hippocampal function, whereas abnormally low or the high levels associated with major stressors impair function [1,7]. In contrast to these detrimental effects of GCs, systemic and intrahippocampal estradiol administration can enhance performance in males and females in spatial and non-spatial memory tasks [8,9,43–45]. Moreover, estrogen rescues both spatial and non-spatial memory from stress-induced learning impairments [20,21].

Here we report a viral vector gene delivery strategy to express a chimeric receptor (ER/GR) that converts the deleterious effects of GCs into protective estrogenic effects. ER/GR is a modular chimeric gene that consists of the C-terminal hormone-binding domain of the glucocorticoid receptor and the N-terminal DNA-binding domain of the primary human estrogen receptor ER α [6,2]. ER/GR, when expressed, has the dual effects of competing with endogenous GR for GCs and converting GR-mediated signaling into neurotrophic ER responses [6]. In this study, we assessed if expression of ER/GR in the dentate gyrus could block the disruptive effects of stress on non-spatial memory. The construction of the ER/GR amplicon vector has been previously described [6]. Briefly, for stereotaxic infusion of vector, rats were anesthetized with 1ml/kg of a mixture consisting of 100 mg/kg ketamine, 10 mg/kg acepromazine, and 100 mg/kg xylazine. ER/GR or a control vector, expressing only a reporter gene GFP, were infused using a Hamilton syringe (Hamilton Company, Reno, NV) with a 28-gauge needle (1 μ l over a 5 min period with a titer of 1×10^6) dorsal to the apex of the dentate gyrus (DG) (coordinates: anteroposterior, 4 mm from bregma; mediolateral, 3.00 from midline;

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dorsoventral, 3.2 mm from dura). Vector was delivered at the coordinates with the use of a microsyringe pump controller (World Precision Instruments, Inc., Sarasota, FL) to ensure uniform delivery. All injections for behavioral studies were bilateral. Animals were housed three to a cage following surgery. Immediately following the behavioral assay, animals were decapitated and perfused transcardially with heparinized saline and a 4% paraformaldehyde (PFA) solution. Brains were sectioned at a 30µm thickness on a cryostat. Granule cells were visualized using a fluorescence microscope at 490nm excitation and 10–20X magnification.

Male Long Evans rats (190–220 g) were housed under standard laboratory conditions on a 12 hr light/dark cycle (lights on at 0700). Animals were immobilized in plastic decapicone bags (Fisher Scientific) and secured at the base of the tail in the dorsal recumbent position to prevent movement for 2 hours/day for 2 consecutive days; immobilization occurred between 1000 – 1200 hours in a neutral cage lined with a diaper pad. Control animals were subjected to gentle handling for five minutes each. Animals were carefully monitored during stress regimens in accordance with the guidelines described by the National Institutes of Health and the Stanford University Department of Veterinary Services and Care. An additional group was subcutaneously injected with estradiol (Sigma-Aldrich Corp. St. Louis, MO) dissolved in peanut oil (15 µg/kg) daily for three days prior to behavioral testing. Subjects were tested on the object recognition task 24hrs after the last injection.

The role of the hippocampus in nonspatial memory is controversial [27–29]; however abundant new data suggest that the hippocampus plays a central role in object recognition performance [30–36]. Testing was performed in an open-field arena (60×60×45) constructed of black Plexiglas. Trials consisted of a sample trial (5 minutes) and a recognition trial (5 minutes). The sample trial and the recognition trial were separated by intertrial intervals of 6hrs and 24hrs. In the sample phase, two identical objects were placed at one end of the open field and amount of time spent exploring the two objects was recorded. For the recognition trial, a new object replaced one of the previous objects. Location and the new object were counterbalanced (left or right) across the trials. Object exploration was scored only when the rat's nose was within 2 cm of the object and the vibrissae were moving. Sessions were videotaped and later analyzed using a stop watch to assess object exploration during the sample and test phase. Student t-tests were used to determine whether time spent with the new object was greater than time spent with the old object. If subjects spent significantly more time exploring the new object, they were considered to have discriminated and remembered.

Expression of control vector, expressing reporter gene (GFP), and experimental ER/GR vector was evident throughout the dentate gyrus subfield of the hippocampus (FIG. 1). The bipromoter vector system employed in this study coexpresses both transgene and reporter gene in a similar pattern, as described previously [16–18]. To assess the protective effects of ER/GR overexpression on non-spatial memory, behavioral testing was performed 3 days post vector infusion, such that peak vector expression occurred around the time of encoding [9]. When discrimination was assessed at the 6hr delay a significant stress effect was observed. Non-stress GFP animals were able to discriminate between old and new objects ($P=0.010$ $n=10$), while stress GFP animals were impaired ($P=.212$ $n=6$) (FIG. 2A). Likewise, non-stress ER/GR animals were able to discriminate ($P=0.037$ $n=6$). However, stress ER/GR animals were impaired ($P = 0.124$ $n=6$) (FIG. 2B). At the 24 hour delay, non-stress GFP animals were able to distinguish between new and old objects ($P=0.002$ $n=10$) (FIG. 3A). However, stress GFP animals were impaired in their ability to discriminate between old and new objects ($P=0.543$ $n=6$) (FIG. 3A). In contrast, both non-stress ER/GR and stress ER/GR animals were able to discriminate ($P = 0.043$ $n=6$, $P = 0.009$ $n=6$ respectively) (FIG. 3B).

The protective effects of ER/GR could be due to blocking GR signaling and/or increasing estradiol signaling. To distinguish between these two possibilities, an experiment evaluating

the protective efficacy of exogenous estradiol was conducted. At the six hour delay, non-stress GFP animals injected with estradiol (GFPE) could discriminate between new and old objects ($P=0.01$ $n=8$), while stress GFPE animals were impaired ($P=0.232$ $n=5$) (FIG. 4A). Conversely, at the 24 hour delay, non-stress GFPE animals were incapable of discriminating between new and old objects ($P=0.769$ $n=8$) (FIG. 4B). In contrast, stress GFPE animals could discriminate, spending significantly more time with the new object ($P = 0.04$ $n=5$) (FIG. 4B).

The use of viral vectors has become an important tool to study and manipulate rodent behavior [15]. Stereotaxic infusion of HSV vectors preferentially infects neurons over glia (85% of neurons, 10–15% glia) up to several millimeters from the infusion site [6,9]. Although infection is confined to a small region, studies show that altering function in this small area is sufficient to influence hippocampus-dependent behavior [46,47].

Glucocorticoids and stress modulate both retrieval and consolidation phases of information processing. For example, the administration of GCs or a stressor immediately post-training enhances the consolidation phase of memory [23]. In contrast, GCs or stress, delivered 30–60 minutes before testing, disrupt the retrieval process [22,46]. Using an immobilization stress regimen we have previously demonstrated impaired performance on the MWM [9]. The present study demonstrates that overexpression of ER/GR effectively blocked the stress-induced impairments of non-spatial memory at the 24 hour delay. We speculate that during the time course of the two day stressor, ER/GR-mediated protection occurs through direct competition with endogenous GR receptors for circulating GCs, thereby lessening detrimental GR-mediated events in DG. Potentially, ER/GR was not protective at the 6hr delay, because stress effects were stronger at 6 hours than 24 hours, and the same level of expression could not overcome stress effects in the former.

Alternatively, the GC mediated activation of ER/GR, may underlie the enhancement in memory consolidation at the 24hr interval. When is ER/GR reducing the effects of stress? We speculate that ER/GR is predominately activated during the course of the two day stressor, when GC levels are elevated. HSV amplicon gene expression starts as early as 4–6 h and peaks at 72 h, which covers the time period of the two day stressor [17]. Moreover, because GR is only heavily occupied during stressors, ER/GR would be as well, meaning that it is only minimally active under basal circumstances.

What mechanisms mediate these effects of ER/GR? BDNF is an excellent marker gene to assess the actions of ER/GR because it is regulated in opposite directions by estrogen and GCs. We have previously demonstrated that a stress-induced activation of ER/GR leads to increased BDNF levels in the dentate gyrus (9). We demonstrated that BDNF mRNA levels were still elevated 3 days post-injection in stressed animals expressing ER/GR, whereas in the absence of stress, no difference in BDNF mRNA expression was found between hemispheres expressing ER/GR or GFP (9). This is supportive of data showing an increase in BDNF mRNA in the dentate granule cell layer 53 hrs after an acute injection of estrogen (57). Numerous studies indicate that both estradiol and BDNF enhance hippocampal-dependent learning in male rats (50,45,37,54). Moreover, BDNF attenuates the impairing effects of stress on hippocampal dependent memory and LTP (11). ER/GR may enhance non-spatial consolidation by increasing BDNF levels which in turn facilitates hippocampal excitability (55). Indeed, BDNF was shown to increase mossy fiber transmission and is critical to the late stage of LTP (55,54).

ER/GR may be conferring protection by attenuating the deleterious effects of glucocorticoids, upregulating the protective effects of estrogen, or both. An estradiol experiment was conducted in order to dissociate ER/GR's estrogenic effects from that of competition with GR for CORT. At the 6 hour timepoint, estradiol was ineffective at blocking the impairing effects of stress on

cognition. In contrast, at the 24 hour delay, non-stress GFPE animals were incapable of performing, whereas stress GFPE animals could discriminate (FIG.4B). This data is supported by the current literature. For example, estrogen enhanced performance in the RAM only in chronically stressed animals but did not affect performance in unstressed controls [21]. The enhanced performance of the stress GFPE animals illustrates the activational effects of estrogen in the context of stress [21]. However, this result should be interpreted cautiously because we cannot state conclusively that the effects of ER/GR on stress are mediated through changes in estrogenic signaling rather than reduction of signaling through endogenous GR. In addition, systemic delivery of estrogen in male rats is not equivalent to ER/GR overexpression in the hippocampus; therefore it is difficult to interpret the results from the estrogen study. Future studies comparing ER/GR overexpression and direct administration of estrogen to the hippocampus should prove informative.

Currently, the effects of estrogen on cognitive function in male rats are not well-defined. However, the literature does suggest that estradiol can enhance cognitive performance in male rodents and humans [37–39,43–45]. Intrahippocampal estradiol administration enhances memory consolidation in both male and female rats [44,45]. In contrast, several studies have found opposite effects or no effects of estradiol treatment in males [40–42].

In conclusion, we demonstrated that overexpression of ER/GR protects against stress-induced impairments of non-spatial memory. The mechanisms that underlie this effect are not entirely clear. However, these findings are supportive of other studies showing that estradiol can enhance cognitive function and protect against the impairing effects of stress on non-spatial memory.

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Figure 1.

Digital images showing robust in vivo expression of GFP-tagged ER/GR transgene 72 hrs post infusion. Expression of both ER/GR and GFP vectors was evident throughout the dentate gyrus subfield of the hippocampus. ER/GR and GFP genes are driven by alpha 4 and alpha 22 promoters, respectively. OriS sits between the promoters and initiates replication. ER/GR is terminated by a human cytomegalovirus (HCMV) polyA and GFP by a simian virus 40 (SV40) polyA signal. A herpes “Pac” sequence is included to provide the packaging signal needed for inclusion of plasmids into viral capsids.

Figure 2.

6 hr object recognition memory during the test phase of the object recognition task. Data represent time spent exploring the old or new object (mean $\pm$ SEM). A, Non-stress GFP animals were capable of distinguishing between objects and spent significantly more time exploring new over old objects ($P = 0.01$ $n=10$). In contrast, stress GFP animals were incapable of discrimination ($P = 0.212$ $n=6$). B, Non-stress ER/GR animals distinguished between new and old objects ($P = 0.03$ $n=6$). Stress ER/GR animals were incapable of discriminating, indicating a strong stress effect ($P = 0.124$ $n=6$). Student t-test NON-STRESS GFP $n=10$, STRESS GFP $n=6$, NON-STRESS ER/GR $n=6$, STRESS ER/GR $n=6$, $*p<0.05$.

Figure 3.

24 hr object recognition memory during the test phase of the object recognition task. Data represent time spent exploring the old or new object (mean $\pm$ SEM). A, Non-stress GFP animals were capable of distinguishing between objects ($P=0.002$ $n=10$), whereas stress GFP animals were incapable of discrimination ($P=0.543$ $n=6$). B, Non-stress ($P=.04$ $n=6$) and stress ($P=0.009$ $n=6$) ER/GR animals were both capable of discriminating between new and old objects. Student t-test NON-STRESS GFP $n=10$, STRESS GFP $n=6$, NON-STRESS ER/GR $n=6$, STRESS ER/GR $n=6$, * $p<0.05$ ** $p<0.01$.

Figure 4.

Object recognition memory during the test phase of the object recognition task for non-stress estrogen (GFPE) and stress estrogen- treated (GFPE) animals. Animals were treated with 15ug/kg of estradiol daily, during the course of the two day stress regimen and tested 24hrs after the last injection. The GFP Data represent time spent exploring the old or new object (mean $\pm$ SEM). A, At the 6 hr delay, non-stress GFPE animals were capable of distinguishing between objects ($P = 0.01$ $n=8$), whereas stress GFPE animals were incapable of discrimination ($P = 0.232$ $n=5$). B, At the 24 hr delay, non-stress GFPE animals could not distinguish between objects ($P = 0.769$ $n=8$). In contrast, stress GFPE animals were capable of discrimination ($P = 0.04$ $n=5$). Paired t-test NON-STRESS GFPE $n=8$, STRESS GFPE $n=5$, * $p<0.05$ ** $p<0.01$