Neuroscience 162 (2009) 863–869

RAPID REPORT

DIFFERENTIAL MODULATION OF HIPPOCAMPAL PLASTICITY IN A NON-NOXIOUS CONFLICT MODEL FOR ANXIETY

D. R. COLLINS

Clinical Sciences Research Institute (UoW Campus), Warwick Medical School, University of Warwick, Gibbet Hill Campus, Coventry, CV4 7AL, UK

Abstract—Modulation of synaptic strength may underlie stress-induced changes in cognitive ability. Long-term memory formation underpinning fear and anxiety behaviors, such as those seen in post-traumatic stress and phobic disorders, is thought to be dependent on amygdalo-hippocampal interactions. In most models, however, painful stimuli are used to induced stress and anxiety. Here, the effects of a novel conflict model, developed to generate a more naturalistic model of anxiety, utilizing two non-noxious stressors (predator (cat) odor and light), on hippocampus plasticity were determined. Exposure to the external stimuli elicited typical, stimulus-specific, anxiety-related behaviors. Dual presentation of the stressors evoked an increase in the variability of behaviors, suggesting that the animals were experiencing conflicting drives. Induction of long-term potentiation (LTP) within the CA1 region of the hippocampus was reduced following exposure to light stress, independent of presence, or absence, of odor. However, after a single presentation, LTP was reduced following either odor presentation or dual presentation of the stressors. Furthermore, LTP in ex vivo tissue obtained from conflict-exposed animals showed differential hemispheric responses, suggesting that long-term contextual-related components of anxiety behavior are dependent on modification of hippocampal circuitry.

Key words: stress, anxiety, behavior, amygdala, hippocampus, LTP.

It is recognized that alterations in long-term memory formation through modulation of synaptic strength may underpin stress-induced changes in cognitive ability (Selden et al., 1991; Izquierdo et al., 1997; Bauer et al., 2001; Seidenbecher et al., 2003). Enhanced activation of amygdala circuitry is associated with stressor exposure (Paré and Collins, 2000; Pape et al., 2005) and amygdala activation has been shown to directly impact on plasticity within the hippocampus (Seidenbecher et al., 2003; Nakao et al., 2004). Modification of synaptic plasticity has been described in both regions following stressor presentation (Rogan et al., 1997; McKernan and Shinnick-Gallagher, 1997; Bauer et al., 2001; Debiec et al., 2002; Rodrigues et al., 2004), suggesting an integrative role for these areas in emotional memory formation (Sapolsky, 2003; Maren and Quirk, 2004; Paré et al., 2004). Furthermore, exposure to stressors can modify plasticity observed in ex vivo tissue (McKernan and Shinnick-Gallagher, 1997; Tsvetkov et al., 2002).

Most conflict models commonly rely on painful stimuli to induced stress and anxiety (Millan and Brocco, 2003), activating pain pathways and eliciting defensive rather than anxiety-related behavior (Blanchard et al., 2001; McGregor et al., 2002; Fendt and Endres, 2008). Here, the effects of a novel conflict model, developed to generate a more naturalistic model of generalized (low-level) anxiety through utilizing two non-noxious stressors: predator odor (McGregor et al., 2002; Takahashi et al., 2008) and light (Bourin and Hascoët, 2003), on synaptic plasticity within the hippocampus were investigated.

EXPERIMENTAL PROCEDURES

Behavioral protocol

All procedures were performed in accordance with the UK Animals (Scientific Procedures) Act 1986 and associated guidelines and approved by the Institutional Ethical Review Committee. All efforts were made to minimize the number of animals used and their suffering. Experiments were performed on 32 male Sprague–Dawley rats (age 26–30 days at commencement), randomly assigned to one of four groups (eight animals per group). Behavioral testing boxes (44.5×30×29 cm) were constructed from Perspex and consisted of a dark "hide" side (13.5×30×29 cm), and a "light" side (30.5×30×29 cm) separated by a panel (white on the light side/black on the hide side) incorporating an exit hole (5.5×6.5 cm). Strips of cotton (25×0.75 cm) were positioned on the back wall of the hide side of the box, 2.5 cm from the floor. The light side of the box was illuminated using either a red light (72/73 lx) or white light (399/400 lx). Animals were habituated to the boxes for 3 days prior to testing under "control" conditions (15 min per day, see below). On test days; animals were exposed to one of four paradigms: "control" (red light, no odor), predator (cat) "odor" (red light), "light" (white light) or "light (white light) plus odor." Strips of cotton impregnated with predator odor were positioned as for habituation (above) in the odor paradigms, control strips were used under "control" and "light" conditions.

Cat odor was utilized as the predator odor and was permeated into the cotton through daily 5–10 min grooming of the cat using cotton gloves for a 2–4 week period and stored in an airtight container at −20 °C until required. On test days strips were handled using non-latex plastic gloves, cut to the required size, positioned on the back wall of the chamber and allowed to warm *in situ* for 15–20 min. Animals were positioned in the centre of the box at the start of the trial and behavior was observed for 15 min and recorded via cameras linked to a computer (utilizing 1/3-inch

Tel: +44-0-2476-574-181; fax: +44-0-2476-574-871.
E-mail address: dawn.collins@warwick.ac.uk (D. R. Collins).
Abbreviations: fEPSP, field extracellular post-synaptic potential; LTD, long-term depression; LTP, long-term potentiation.

0306-4522/09 $ - see front matter
doi:10.1016/j.neuroscience.2009.06.020

B/W microcameras linked to the GeoVision GV600 video surveillance system, GeoVision UK Ltd., Milton Keynes, UK) for off-line analysis. Animals were tested for 3 consecutive days and then subjected to a retrial session following a 3 day rest period, all retrial tests were performed as for test days.

Behavior was analyzed visually and four behaviors typically associated with anxiety and stress-related states were utilized in this study: light/dark ratio, strip removal times, flat-back approach, freezing and rearing behavior (for examples postures see Fig. 1 insets and Dielenberg and McGregor, 2001). Time spent in the "light" side of the box (light/dark ratio), in flat-back approach, freezing and in rearing behavior is presented as percentage total time (900 s). The time taken for removal of the cotton strip from its position on the back wall of the hide area is presented in seconds.

Single exposure experiments

A further set of experiments aimed to determine the effect of short-term exposure to the behavioral protocol was carried out. Experiments were performed on 20 male Sprague–Dawley rats (as above), randomly assigned to one of the four groups (four animals each for the "control" and "odor" groups, six animals for the "light" and "light plus odor" groups). Following habituation, as above, animals were exposed to "control" (red light, no odor), predator (cat) "odor" (red light), "light" (white light) or "light (white light) plus odor" conditions for one 15 min run. Within 2 h of behavioral exposure, tissue was removed for ex vivo electrophysiological testing (see below).

Ex vivo electrophysiological recordings

Within 48 h of retrial testing animals were terminally anaesthetized with isoflurane, decapitated and 400 μm thick transverse hippocampal slices prepared. In order to reduce introduction of time-dependent bias into the data, animals were culled such that each group was represented equally over the time distribution (two animals per day).

In the subsequent set of single exposure experiments animals were culled following the initial exposure to the test condition, within 2 h of behavioral testing.

Slices were maintained in a constantly perfused, humidified interface chamber at 29–30 °C. Artificial cerebrospinal fluid for perfusion contained (in mM): NaCl 124, KCl 3, NaH_2PO_4 1.25, $MgSO_4$ 1, $NaHCO_3$ 26, $CaCl_2$ 2 and D-glucose 10, equilibrated to pH 7.4 with 95% O_2/5% CO_2. Field excitatory post-synaptic potentials (fEPSPs) were recorded from the CA1 region using glass micropipettes (containing 3 M NaCl, resistance 3–5 MΩ) in response to stimulation of the Schaffer–collateral commissural pathway (0.033 Hz, 0.8–20 V), using bipolar stimulating electrodes (FHC Inc., Bowdoin, ME, USA). Following stabilization of the fEPSP (set at 30%–40% maximum amplitude), long-term potentiation (LTP) was induced by application of a single train of stimuli (tetanus, 100 Hz/500 ms at control voltage, time 0). Potentiation of the fEPSP amplitude was observed for at least 40 min post-tetanus.

For all Experiments 2–4 slices were obtained from each animal, yielding one to two recordings from each hemisphere, per animal. Variability in number of slices utilized for each group is a result of strict quality control, in which experiments where the fEPSP recording did not stabilize within an hour of commencing recording (for a minimum of 10 min prior to application of the tetanic stimulus), or exhibiting artefacts mid experiment, were discarded.

Statistical analysis

Data were analyzed using InStat (GraphPad Inc.), utilizing Student's *t*-test, repeated measures ANOVA with Tukey–Kramer multiple comparisons test or one-way ANOVA as appropriate; $P<0.05$ was considered significant. Full details of behavioral analysis and ANOVA results are presented in Table 1. Data are presented as mean±SEM.

RESULTS

Model exposure induced typical anxiety-related behaviors

Under "control" conditions animals spent an average of 30.28±2.85% of trial time in the light area of the box on test days. Control (no odor) strips of cotton were physically removed from the hide area wall in 94% of trials, within 153.6±34.2 s of trial commencement ($n=8$). Exposure to "odor" conditions evoked rapid and persistent changes in behavior; following oronasal contact with odor-impregnated strips. Animals spent significantly increased amounts of time in the light area (64.17±14.52%, $n=8$, $P<0.001$, repeated measures ANOVA; see Table 1). Strip removal occurred in 22% of trials (removal time was 742.4±39.7 s, $n=8$, $P<0.001$, repeated measures ANOVA, Fig. 1). Under "light" conditions animals spent 8.58±0.57% of time in the light area and strips were removed in 97% of runs. With dual "light plus odor" presentation, animals displayed variable, inconsistent behavior; spending 13.24±3.57% of time in the light side ($n=8$, n.s., Fig. 1); strips were removed in 25% of trials and freezing behavior was pronounced. Under "light plus odor" conditions animals spent 15.57±1.19% of time exhibiting freezing behavior, compared to 9.71±0.45% in "odor," 1.47±0.09% in "light" and 0.18±0.05% under "control" conditions ($n=8$/group, $P<0.001$, repeated measures ANOVA, Fig. 1). Behaviors persisted over test and retrial days; however, there was notable variation in rearing behavior. No significant group differences were observed between groups (see Table 1). Significant differences between "odor" and "light plus odor" groups were observed for time spent in the light area and flat-back approach ($P<0.001$ and $P=0.021$ respectively, repeated measures ANOVA, Fig. 1), but not for strip removal and rearing behavior.

Single exposure to the model modifies behavior

A subsequent set of experiments determined the effect of a single exposure to the four conditions. Behavioral data from the single exposure experiments were consistent with the observations noted above. The time taken for removal of the cotton strip was significantly reduced in the presence of predator odor (removal time was 173.33±55.52 s under "control" conditions ($n=4$); 132.17±19.30 s under "light" conditions ($n=6$); 737.50±122.84 s under "odor" conditions ($n=4$) and 900±0 s under "light plus odor" conditions ($n=6$); $P<0.0001$, one-way ANOVA). Time spent in the light area of the box was also altered as for the repeated exposure experiments. Under "odor" conditions animals spent an increased amount of time in the light area of the box (52.07±16.18% of time, $n=4$) compared to control (20.47±8.03%, $n=4$); the presence of light, independent of presence of odor causing a significant reduction in the time spent in the light area (0.83±0.51% of time under "light," $n=6$, and 0.32±0.29% under "light plus odor" conditions. $N=6$). The presence of "odor" led to a significant increase in time spent in both flat-back approach ($P=0.0008$, one-way ANOVA; animals spent 2.61±0.81% of time in flat-back behavior under "odor," $n=4$; 0.50±0.28% of time under "light