

# Repeated Social Stress Increases Reward Salience and Impairs Encoding of Prediction by Rat Locus Coeruleus Neurons

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Stress is implicated in psychopathology characterized by cognitive dysfunction. Cognitive responses to stress are regulated by the locus coeruleus–norepinephrine (LC–NE) system. As social stress is a prevalent human stressor, this study determined the impact of repeated social stress on the relationship between LC neuronal activity and behavior during the performance of cognitive tasks. Social stress-exposed rats performed better at intradimensional set shifting (IDS) and made fewer perseverative errors during reversal learning (REV). LC neurons of control rats were task responsive, being activated after the choice and before reward. Social stress shifted LC neuronal activity from being task responsive to being reward responsive during IDS and REV. LC neurons of stressed rats were activated by reward and tonically inhibited by reward omission with incorrect choices. In contrast, LC neurons of stress-naïve rats were only tonically inhibited by reward omission. Reward-related LC activation in stressed rats was unrelated to predictability because it did not habituate as learning progressed. The findings suggest that social stress history increases reward salience and impairs processes that compute predictability for LC neurons. These effects of social stress on LC neuronal activity could facilitate learning as indicated by improved performance in stressed rats. However, the ability of social stress history to enhance responses to behavioral outcomes may have a role in the association between stress and addictive behaviors. In addition, magnified fluctuations in LC activity in response to opposing behavioral consequences may underlie volatile changes in emotional arousal that characterize post-traumatic stress disorder.

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In response to acute stressors, neural systems that regulate the hypothalamic–pituitary–adrenal axis, autonomic function, behavior, and cognition are engaged in a coordinated manner to cope with the stressor and promote survival. Persistent or repeated activation of these stress systems as a result of chronic or repeated stress, or inappropriate activation of the systems in the absence of stress, is maladaptive and results in pathology (Chrousos and Gold, 1992; de Kloet *et al*, 2005; McEwen, 1998). Such maladaptive stress responses are thought to underlie symptoms such as hyperarousal, inappropriate fear, and attentional and cognitive dysfunctions that characterize certain psychiatric disorders (Gold and Chrousos, 2002). Interestingly, although many studies report impairment of cognitive processes by stressors, there are also reports of enhancement and these differences may depend on the specific stressor and the cognitive endpoint (Bondi *et al*, 2008; Butts *et al*, 2013; Graybeal *et al*, 2011; Lapid-Bluhm *et al*, 2009; Thai *et al*, 2013). These differences underscore the complexity of effects of stress on neural circuits that regulate cognitive functions.

The locus coeruleus–norepinephrine (LC–NE) system is a stress-response system that has been implicated in cognitive responses to stress and in stress-related psychopathology (Southwick *et al*, 1999; Valentino and Van Bockstaele, 2008; Wong *et al*, 2000). LC neurons are spontaneously active and their discharge rate is positively correlated to behavioral indices of arousal (Aston-Jones and Bloom, 1981b; Foote *et al*, 1980). Salient stimuli elicit a phasic activation of LC neurons that precedes orientation to the stimulus and this has implicated the system in attention (Aston-Jones and Bloom, 1981a; Berridge and Waterhouse, 2003; Foote *et al*, 1980). LC neuronal recordings in animals performing operant tasks indicate that phasic LC discharge is associated with focused attention and staying ‘on-task’, whereas high-tonic LC discharge is associated with labile attention and task disengagement (Aston-Jones and Cohen, 2005; Bouret and Sara, 2005; Sara, 2009). It has been suggested that this high-tonic mode of LC discharge facilitates cognitive flexibility (Aston-Jones and Cohen, 2005). Acute stressors and exposure to the stress-related peptide, corticotropin-releasing factor (CRF) bias LC activity towards a high-tonic state that may be adaptive in a dynamic environment with life-threatening challenges (Curtis *et al*, 2012; Valentino and Foote, 1987; Valentino and Wehby, 1988). Notably, certain doses of CRF improve extradimensional set-shifting performance during an attentional set-shifting task (AST), an endpoint of cognitive flexibility (Snyder *et al*, 2012). In contrast to the activating effects of acute stressors on

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LC neuronal activity, repeated social stress produces an enduring inhibition of rat LC discharge that is apparent several days after the last stressor (Chaijale *et al*, 2013). These findings are relevant to humans given that social stress is one of the most common human stressors. A similar enduring inhibition of LC activity occurs in rats exposed to a model of post-traumatic stress disorder that involves the administration of three consecutive stressors (George *et al*, 2013). It is unknown how this state of LC inhibition impacts on cognition.

As cognitive dysfunction is thought to be one of the maladaptive consequences of repeated stress and the LC-NE system has been implicated in cognitive aspects of the stress response, the present study investigated the long-term effects of repeated social stress on LC activity recorded during the performance of an AST that assesses simple discrimination (SD), compound discrimination (CD), intradimensional set shifting (IDS), reversal learning (REV), and extradimensional set shifting. The results provided evidence that repeated social stress changes the relationship between LC neuronal activity and task performance, and renders LC neurons responsive to reward regardless of its predictability.

## MATERIALS AND METHODS

### Animals

Adult male Sprague–Dawley rats were used as intruders or matched controls (275–300 g) and male Long–Evans (650–850 g) retired breeders were used as residents (Charles River, Wilmington, MA). Rats were individually housed in a controlled environment (20 °C, 12 h light/dark cycle, lights on at 0700 h). Food and water were available *ad libitum* unless noted. Care and use of animals was approved by the Children's Hospital of Philadelphia Institutional Animal Care and Use Committee and in accordance with NIH Guide for the Care and Use of Laboratory Animals.

### Surgery and Stress

Figure 1a1 shows an overall timeline of the experimental protocol. All rats used as intruders or matched controls were anesthetized, positioned in a stereotaxic instrument, and surgically prepared for localization of the LC and subsequent implantation of an eight-wire microelectrode array for neuronal recordings as previously described (Curtis *et al*, 2012). Following recovery, rats were randomly assigned to receive control manipulation or social stress in the resident–intruder model of social stress (Chaijale *et al*, 2013; Miczek, 1979). Intruder rats were placed into the cage of a resident rat. A period of investigation was typically followed by an aggressive encounter initiated by the resident. When the intruder either assumed a supine posture signaling subordination (defeat) or 15 min had elapsed from the time when the intruder was placed into the cage, the intruder and resident were separated by a wire mesh partition to prevent further physical contact for the remainder of a 30-min session. Matched control rats were placed into a novel cage for the same duration (Chaijale *et al*, 2013; Wood *et al*, 2010).

### Attentional Set-Shifting Task

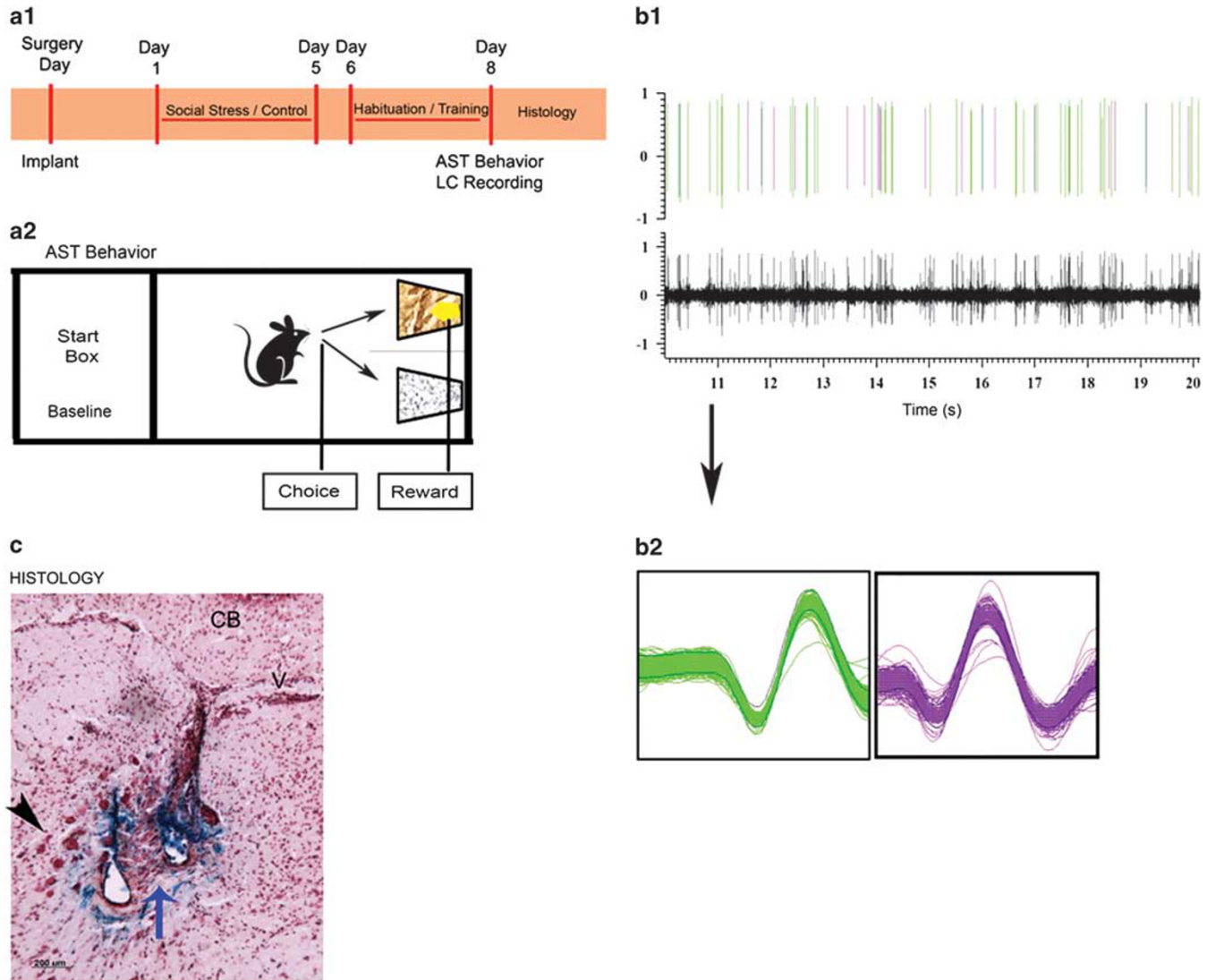
Three days after the start of the experimental manipulation, rats were food restricted to 85% of their free-feeding weight. After the fifth day, rats were trained to perform an AST task (Figure 1a2; AST), which consisted of 1 day of habituation and 1 day of training as previously described (Birrell and Brown, 2000; Snyder, *et al*, 2012). Testing occurred 3 days after the last experimental manipulation and consisted of five discrimination stages: (1) SD, in which the rat chose to dig in one of two pots containing different digging materials to find food reward; (2) CD, which required the same discrimination as before, however, the pots were scented with different odors, thereby providing irrelevant stimuli from a new dimension; (3) IDS, which changed exemplars from each dimension but maintained the same rule as SD so that the digging material was the relevant dimension; (4) REV, in which the reinforcement was associated with the alternate stimulus from the same dimension as IDS; (5) extradimensional shift (EDS), in which exemplars were changed and the relevant cue was changed to the other sensory dimension (odor). After the completion of each trial, rats were placed back into the start box. Six consecutive correct trials were required to proceed to the next stage. Behavior was videotaped and synchronized with the electrophysiological recording. Performance was quantified as the mean number of trials to reach criterion. In addition, analysis of error type during REV was quantified and broken down into perseverative and regressive errors as previously described (Cao *et al*, 2012).

### Electrophysiology

The implanted multiwire array was connected by cables to the data acquisition system (Alpha Omega, Alpharetta, GA) and LC discharge rate was recorded before the start of the test and throughout testing as previously described (Curtis *et al*, 2012). LC discharge was recorded in the start box of the arena before starting each trial for at least 30 s (Figure 1a2; Baseline) and during task performance. The cable was connected at all times and rats were able to freely move during AST performance. Multiunit activity on all eight wires (channels) was monitored in real time and spike sorting of multiunit activity was done offline (Curtis *et al*, 2012).

### Analysis

The WaveMark template-matching algorithm in the Spike2 software (Cambridge Electronic Design, CED, v7.09) was used to discriminate putative LC single-unit waveforms. A set of waveforms identified by the WaveMark template is verified as events from a single unit by the principal component analysis and associated autocorrelograms (Figure 1b1 and b2). Of 12 control and 10 stressed rats that were implanted with multichannel electrodes in the LC, well-discriminated single units were obtained from 3 rats of each group by the time the rats were tested in the AST. For each channel with LC activity, two to five single units were usually discriminated. LC activity was typically isolated on two to four channels in an individual rat. LC discharge rates from all cells from each treatment group were averaged and



**Figure 1** Schematic of experimental protocol. (a1) Timeline of surgery, stress, behavioral training, testing, and recording: Post surgery, rats were exposed to 5 days of social stress or control manipulation followed by habituation (Day 6) and training (Day 7) in the attentional set-shifting task (AST). Testing and locus coeruleus (LC) recording occurred on Day 8. (a2) Rats were placed in the start box and LC discharge was recorded before each trial of the task (baseline). The partition was removed for the animal to start the trial. The rat would travel to the other end of the arena where it chose to dig in one of two pots (separated by a partition) for food reward. LC activity was recorded before rats commit to a particular pot (choice), during the time between choice and reward, or reward omission (behavioral outcome), and after the behavioral outcome. (b1) The bottom trace shows raw waveforms from a single microwire located in the LC. The top trace shows sorted units from discriminated LC waveforms shown in b2. (b2) Waveforms are discriminated and analyzed off-line (depicted by the arrow). Waveforms from these units exhibited separable clusters when plotted in principal component analysis. (c) Photomicrograph illustrating the final placement of two of eight microwires in the LC as depicted by the blue arrow (black arrowhead, mesencephalic nerve 5; V, ventricle; CB, cerebellum).

plotted in 1-s. bins. The electrophysiological analysis focused on LC activity during IDS, REV, and EDS only, because behavior was identical between treatment groups for SD and CD, and there were few incorrect trials in these stages. As cognitive processes and the neural circuits involved in IDS, REV, and EDS are different, LC activity during the individual stages was analyzed independently (Robbins and Roberts, 2007). Mean LC activity for all cells recorded in a treatment group (control vs stress) during IDS, REV, or EDS was analyzed over the last six correct and the preceding six incorrect trials to correspond to the time that the rule was learned. In addition, for REV, activity was analyzed for the initial trials when the contingency was first

reversed. For each trial, LC activity was analyzed at baseline (in the start box). Throughout the trial, LC activity was synchronized to the behavioral outcome (reward or reward omission). The average time between the choice (when animals committed to dig in one specific pot) and behavioral outcome was also determined. A one-way repeated-measures ANOVA was used to determine whether LC activity changed across time during the performance of a particular task stage over a period from 5 s before the choice to 10 s after behavioral outcome. *Post-hoc* comparisons (Student–Neuman–Keuls test) were done to determine at what time points during this period LC activity differed from the 5-s pre-choice baseline (SigmaStat, Systat

software). In addition, to analyze whether stress induced phasic changes in reward-related neuronal activity, spike counts were reanalyzed in 50 ms bins for 2 s before and 2 s after the outcome. For each cell, the *z*-score was calculated by normalizing the mean spike count from each bin to the average activity of that unit during baseline. Heatmaps were generated to represent the mean *z*-score activity from each treatment group for the last six correct and preceding six incorrect trials. Values were smoothed with a 5-point averaging filter. In addition, histograms were generated to compare the activity of all cells from each treatment group across trials. Specifically, *z*-score values for the last six correct and preceding six incorrect trials were collapsed and plotted between a period of 2 s before and 2 s after reward or reward omission, and a paired Student's *t*-test was used for statistical comparison. Finally, to analyze LC phasic activation in response to the behavioral outcome during REV, the percentage of units firing simultaneously were calculated for every 50 ms bin. Results were plotted as heatmaps and time locked as 2 s before and 2 s after reward or discovery of reward omission (for incorrect trials). Heatmaps representing LC neurons from one control and one stressed rat were plotted with a set threshold such that bins would represent firing from at least 50% of the units. When at least 50% of the total number of cells from each rat fired within the same 50 ms bin, it was considered that LC activity was phasically activated. The non-parametric Wilcoxon signed-rank test was used to compare phasic activation before and after behavioral outcome.

## Histology

Recording sites were localized by passing current (10  $\mu$ A, 15 s) through the wires followed by transcardial perfusion with 6% potassium ferrocyanide in 0.1 M phosphate buffer to form a Prussian blue reaction (Curtis *et al*, 2012). After perfusion, brains were removed and stored in  $-80^{\circ}$ C. Frozen coronal sections (30  $\mu$ m) were cut through the LC and thaw-mounted onto slides, stained with Neutral red, and the recording site

was identified by microscopic visualization of the Prussian blue reaction product (Figure 1c).

## RESULTS

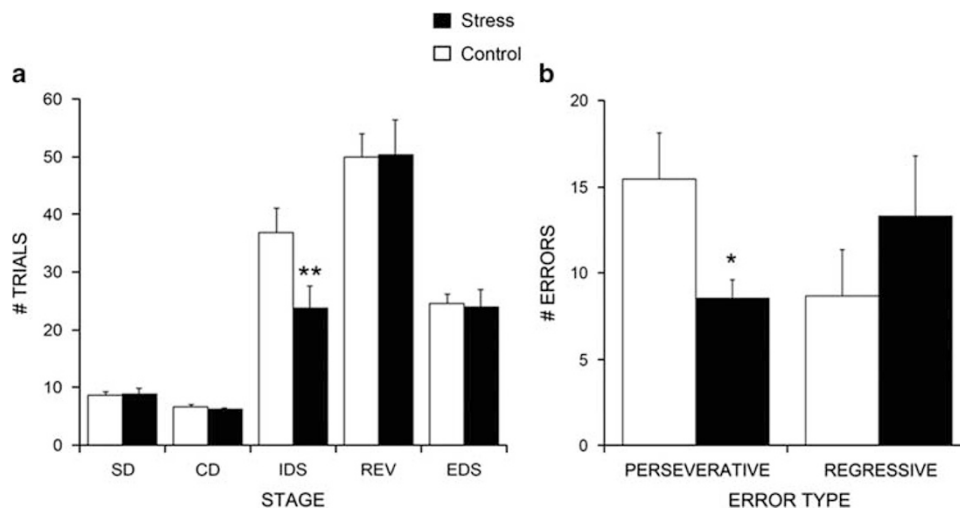
### Social Stress History Improves Performance in Certain Stages of the AST

Performance during most AST stages was comparable between control ( $n=12$ ) and stressed ( $n=10$ ) rats based on the mean number of trials to reach criterion (Figure 2a). However, stressed rats performed better during the IDS stage of the task (Figure 2a). A repeated-measures ANOVA indicated an interaction that was driven by IDS performance ( $F(4,79)=7.0$ ,  $p<0.01$ ). Based on trials to criterion, REV performance was comparable between stressed and control groups. However, analysis of error type revealed that stressed rats made fewer perseverative errors ( $p<0.05$ , Student's *t*-test; Figure 2b). Although this appeared to be accompanied by an increase in regressive errors, this was not statistically significant ( $p=0.3$ ).

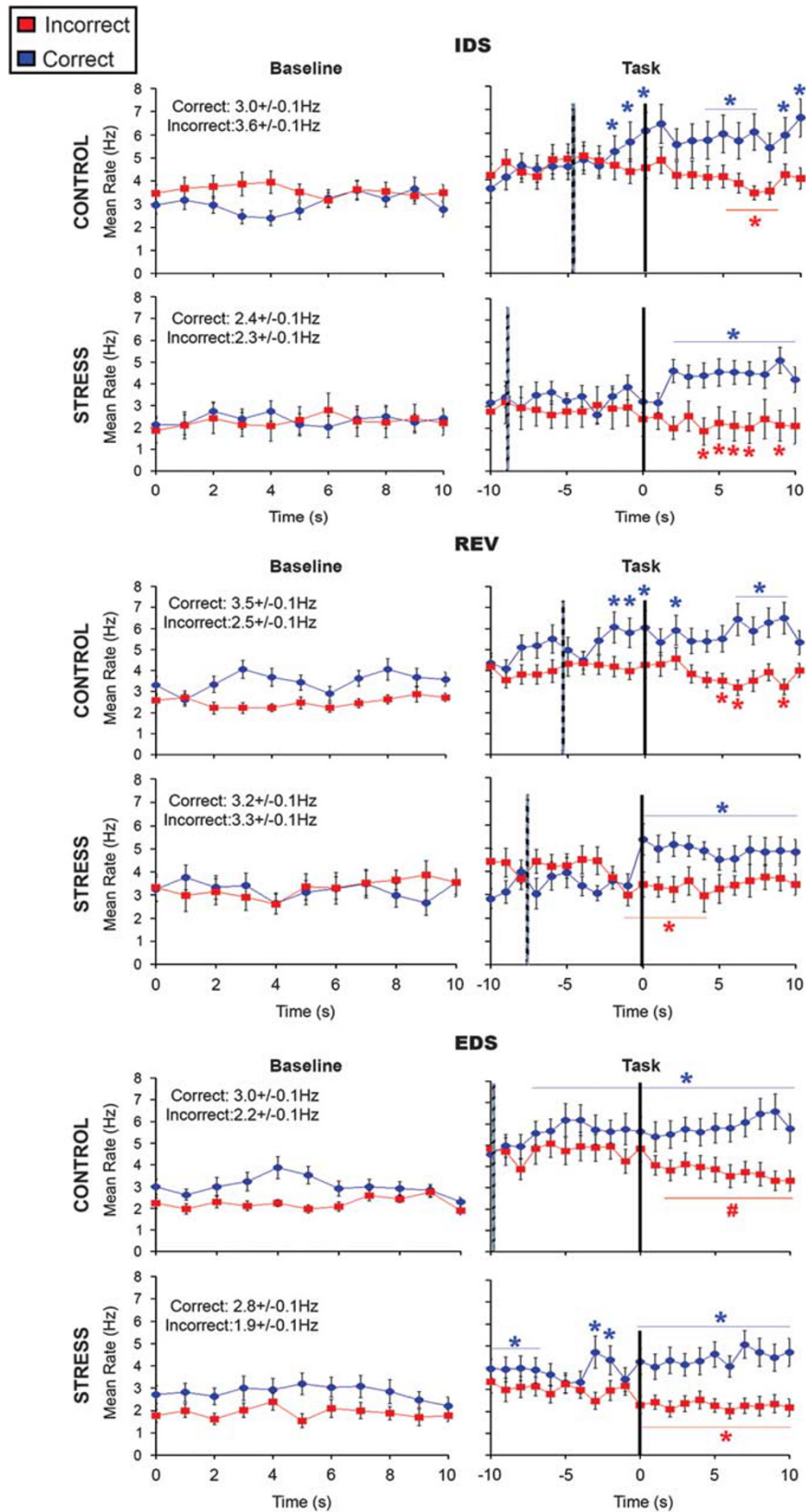
The duration between the time at which rats chose a pot and the time at which the outcome (reward or reward omission) was detected was calculated for the last six correct and incorrect trials of IDS, REV, and EDS. Rats with a history of social stress showed a greater delay between choice and outcome ( $11.0 \pm 1.1$  s) compared with control rats ( $7.1 \pm .5$  s,  $p=0.02$ , Student's *t*-test). Supplementary Figure S1 shows these data for individual stages and further divided into incorrect and correct trials. This effect is apparent in the graphs in Figure 3 (Task) that are synchronized to the outcome and also show the average time at which the choice occurred before the outcome for each group in each task.

### Social Stress History Renders LC Neurons Less Task Responsive and More Reward Responsive

All control ( $n=12$ ) and stressed ( $n=10$ ) rats tested in the behavioral task described above were implanted with



**Figure 2** Attentional set-shifting performance of control and stressed rats. (a) The abscissa indicates the task stage: simple discrimination (SD), compound discrimination (CD), intradimensional shift (IDS), reversal learning (REV), and extradimensional shift (EDS). The ordinate indicates the number of trials to reach criterion. Bars represent the mean performance of 12 control (open bars) and 10 stressed (solid bars) rats  $\pm$  SEM. (b) The abscissa indicates the error type during reversal learning (REV) and the ordinate indicates the number of errors. Bars are mean  $\pm$  SEM \* $p<0.05$  and \*\* $p<0.01$ .



**Figure 3** Locus coeruleus (LC) neuronal activity varies with behavior during intradimensional set shifting (IDS), reversal learning (REV), and extradimensional set-shifting (EDS) performance. Graphs on the left show 10 s of LC spontaneous activity (Baseline), for control and stressed rats, before starting each correct (blue) or incorrect (red) trial. Mean LC discharge rates are provided in the insets. Graphs on the right represent mean LC activity during the last six correct (blue) and last six incorrect (red) trials, during IDS, REV, and EDS task performance (Task). LC activity is time synchronized with reward or reward omission (solid line at  $t = 0$  s). Dashed lines represent the average time at which the choice was made based on the mean duration between choice and reward. LC discharge is expressed as mean discharge rate across all units (mean  $\pm$  SEM). \* $p < 0.05$ , Student–Neuman–Keuls.

multichannel electrodes in the LC. Of these, 3 control (20 units) and 3 stressed (19 units) rats yielded well-discriminated single unit LC recordings after 5 days of social stress and 3 days of training and testing. Before testing, mean LC discharge rates were  $3.9 \pm 0.4$  Hz and  $2.4 \pm 0.3$  Hz for control and stressed rats, respectively ( $p < 0.01$ , Student's *t*-test). This was consistent with previous reports that baseline LC discharge rate is lower in rats with a history of social stress (Chaijale *et al*, 2013). During the last six correct and previous six incorrect trials baseline LC discharge recorded in the start box was relatively stable for both treatment groups and in all task stages (Figure 3, left-hand panels). A comparison of the baselines before starting the IDS stage showed no significant differences between correct and incorrect trials for either controls or stressed ( $p > 0.05$ , Student's *t*-test) groups. In contrast, analysis of REV and EDS baselines demonstrated that LC neuronal activity was lower when control rats were about to start incorrect trials as compared with correct trials ( $p < 0.05$  for both REV and EDS). This effect was not observed in comparing LC baseline activity from stressed rats during any task stage (Figure 3 'Baseline').

For each task stage, LC neuronal activity varied with specific trial components in a way that differentiated treatment groups (Figure 3, Task). For control rats during correct IDS trials, LC activity increased ( $F(20,419) = 6.9$ ,  $p < 0.001$ , one-way repeated-measures ANOVA) and *post-hoc* analysis indicated that this increase occurred during the period between choice and reward, compared with a 5-s pre-choice baseline, and did not increase further after the reward outcome (Figure 3, IDS, Task, Control). LC activity also varied during incorrect IDS trials ( $F(20,419) = 3.5$ ,  $p < 0.001$ , one-way repeated-measures ANOVA). However, during incorrect trials, LC discharge rate significantly decreased after discovery of reward omission (Figure 3, IDS, Task, Control). This decrease was only apparent at a few time points (6–8 s after outcome).

Similar to that of control rats, LC activity of stressed rats changed during correct ( $F(23,455) = 7.5$ ,  $p < 0.001$ , one-way repeated-measures ANOVA) and incorrect ( $F(23,455) = 2.4$ ,  $p < 0.001$ , one-way repeated-measures ANOVA) trials of IDS performance (Figure 3 IDS, Task, Stress). However, in contrast to control rats, LC activity was not altered from the pre-choice baseline during the period between choice and reward for stressed rats during either correct or incorrect trials. Rather, LC activity of stressed rats were uniquely responsive to the behavioral outcome, being increased after reward for correct trials and inhibited after the discovery of reward omission for incorrect trials.

During REV, a similar distinction was apparent between control and stressed rats in the point at which LC activity was altered during performance. For control rats, LC discharge rates increased during correct REV trials ( $F(20,419) = 4.9$ ,  $p < 0.001$ ) and this occurred during the period between choice and reward as compared with a 5-s pre-choice baseline, and was not increased further in response to reward (Figure 3 REV, Task, Control). LC discharge rate decreased with the reward omission during incorrect trials ( $F(20,419) = 2.9$ ,  $p < 0.001$ ) but this was only statistically significant for a few time points between 5 and 9 s after the outcome. For stressed rats, LC neurons were clearly activated in response to reward ( $F(22,398) = 8.1$ ,

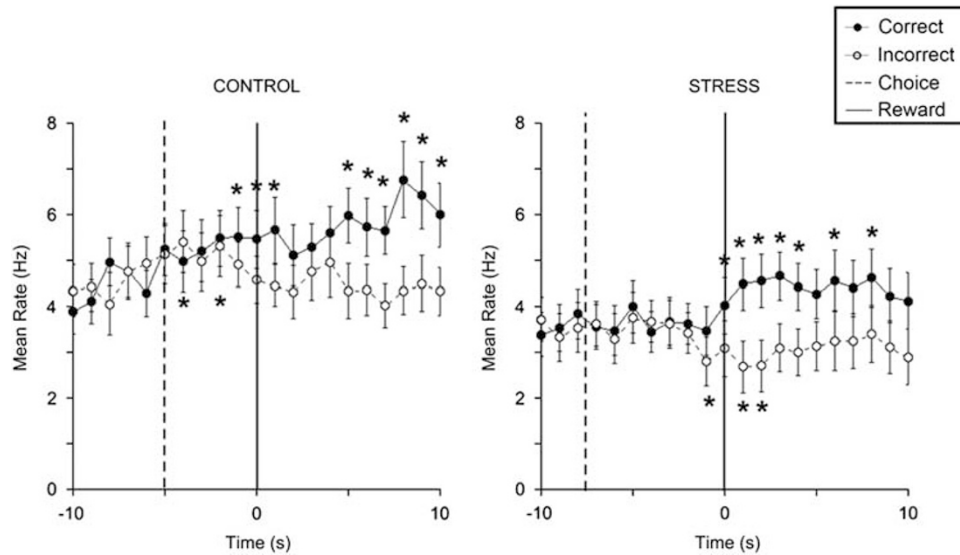
$p < 0.001$ ) but not during the period between choice and reward (Figure 3, REV, Task, Stress). As for control rats, LC activity of stressed rats was decreased by reward omission during incorrect trials ( $F(20,398) = 3.6$ ,  $p < 0.001$ ) (Figure 3, Task, REV).

As LC responses to reward are related to predictability (Bouret and Sara, 2004), LC activity during the initial trials of REV, when contingencies were first reversed, was also examined. The relationship between LC activity and behavior during these early trials was similar to that seen in later trials (Figure 4). LC discharge rates of control rats increased during performance ( $F(20,419) = 6.4$ ,  $p < 0.001$ ), and this occurred specifically during the choice-to-reward period and remained increased compared with the 5-s pre-choice baseline. LC discharge rates of stressed rats were also increased during task performance but only after reward ( $F(23,455) = 6.4$ ,  $p < 0.001$ ). During the incorrect trials, LC activity of control rats increased during choice-to-reward period ( $F(20,419) = 2.5$ ,  $p < 0.001$ ) but did not change after reward omission. For stressed rats during incorrect early REV trials, LC activity decreased just before the discovery of reward omission and this was maintained for a few additional seconds ( $F(23,455) = 2.6$ ,  $p < 0.001$ ).

Similar to the previous stages, during EDS LC neuronal activity of control rats changed during task performance ( $F(24,499) = 6.7$ ,  $p < 0.001$ ) and was specifically increased within the period between choice and reward compared with a 5-s pre-choice baseline and did not increase further with reward (Figure 3 EDS, Task, Control). There was only a trend for LC activity to be inhibited by reward omission during incorrect trials ( $F(24,499) = 1.5$ ,  $p = 0.07$ ). Notably, LC neurons of stressed rats responded in a similar manner as control rats during EDS in that they were activated during the choice-to-reward component compared with the 5-s pre-choice baseline and were not further responsive to reward during EDS ( $F(29,569) = 6.3$ ,  $p < 0.001$ ). In addition, the neurons were inhibited by reward omission during incorrect trials ( $F(29,590) = 4.6$ ,  $p < 0.001$ ) (Figure 3 EDS, Task, Stress).

### LC Neurons of Stressed Rats are Phasically Activated by Reward During REV

LC discharge was further examined across a more discrete time frame ( $\pm 2$  s) around the behavioral outcome to determine whether LC neurons were phasically activated by reward. Figure 5a shows LC activity for controls and stressed rats during correct and incorrect REV trials with respect to behavioral outcome expressed as *z*-score transformations normalized to baseline rates (see Figure 3). For REV, the results seen within this discrete time frame reflect those seen in the longer time frame in Figure 3. LC activity of control rats is activated above baseline and remains constant even after reward. For stressed rats, LC activity is not increased before reward but is abruptly increased after reward for about 800 ms ( $p < 0.05$ , Student's paired *t*-test). The absence of reward had no immediate consequence on LC discharge of either stressed or control rats (Figure 5a). Phasic LC activation elicited by reward did not occur during IDS or EDS components of the task.



**Figure 4** Mean firing rate of locus coeruleus (LC) neurons from control and stress rats during early trials of reversal learning (REV). Graphs represent mean LC mean firing rate (Hz) during the first six correct (solid circles) and first six incorrect (open circles) trials of REV. Discharge rate is time-synchronized to the reward point (solid line,  $t = 0$  s). Dashed lines indicate the average time before outcome that the choice occurred. LC discharge is expressed as mean discharge rate across all units (mean  $\pm$  SEM). \* $p < 0.05$ , Student–Neuman–Keuls.

To visualize phasic activation on a trial-by-trial basis, heatmaps of the normalized ( $z$ -score) LC activity recorded during the last six correct trials and previous six incorrect trials during REV performance were generated (Figure 5a and b, respectively). The heatmaps reflect the results depicted in the histograms and there was no apparent effect of trial order.

Differences in reward-related phasic LC activity during REV were also apparent in heat maps illustrating the percentage of the total number of cells that fired within the same 50 ms bin, (Figure 5b). LC phasic activation associated with reward is apparent in the stressed rat ( $p < 0.05$ , paired Student's  $t$ -test; Figure 5, Stress, Correct) but not the control rat (Figure 5, Control, Correct). There are no apparent changes in LC activity by reward omission.

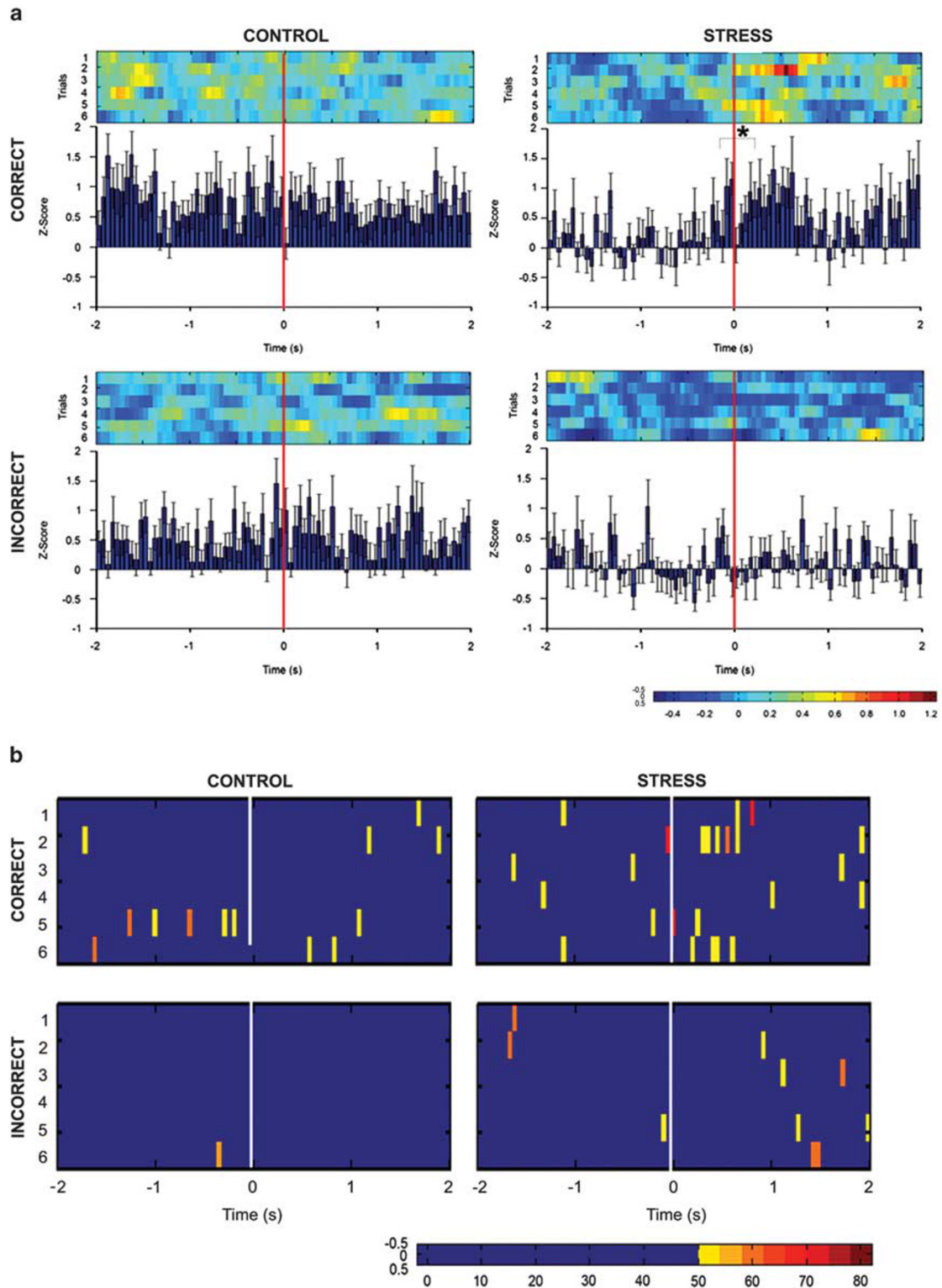
## DISCUSSION

Although stress has generally been associated with impaired cognitive function, here a history of repeated social stress was associated with better IDS performance and decreased perseverative responding during REV. Importantly, social stress changed the relationship between LC discharge rate and behavior. In agreement with previous studies (Bouret and Sara, 2004; Clayton *et al*, 2004), LC neurons of control but not stressed rats were task-responsive for correct trials, exhibiting greater discharge rates between choice and the behavioral outcome. This was associated with a shorter time to obtain reward after making the decision for control compared with stressed rats. Social stress history shifted LC neurons from being task responsive to being reward responsive during correct trials. LC responses to reward were unrelated to prediction error, because they did not habituate during later trials in which reward would be expected. LC neurons of both experimental groups were

inhibited by reward omission in incorrect trials. These results suggest that social stress increases reward salience and impairs encoding of predictability by LC neurons. Stress-induced changes in the relationship between LC neuronal activity and aspects of goal-directed behavior could facilitate certain types of learning and protect against perseverative behavior. However, this stress-induced plasticity may also be expressed as exaggerated arousal responses to behavioral consequences that are characteristic of psychiatric disorders, such as PTSD. The increased reward salience is also seen in humans exposed to stress and this may have a role in the link between stress and addictive behaviors (Mather and Lighthall, 2012).

## Repeated Social Stress Enhances Some Aspects of Cognitive Performance

Both acute and chronic stressors have been demonstrated to produce impairments of executive function and this has been linked to effects on the prefrontal cortex (PFC) (Arnsten, 2009; Holmes and Wellman, 2009). Thus, acute tail pinch, chronic restraint, and chronic unpredictable stress impair attention set-shifting or strategy-shifting performance (Bondi *et al*, 2008; Butts *et al*, 2013; Jett and Morilak, 2013; Liston *et al*, 2006). However, this effect does not generalize across all stress conditions. For example, chronic cold stress selectively impaired REV (Lapiz-Bluhm *et al*, 2009). Moreover, acute restraint and repeated (but not a single) swim stress enhanced REV (Graybeal *et al*, 2011; Thai *et al*, 2013). It has been suggested that by decreasing the influence of the ventromedial PFC, stressors bias the regulation of cognitive processes toward systems that favor alternate strategies, such as the orbitofrontal cortex and dorsolateral striatum, and this may explain the enhancement of REV by some stress conditions (Graybeal *et al*, 2011). In this light, in the present study a history of repeated



**Figure 5** Stress increased phasic locus coeruleus (LC) responses to reward during reversal learning (REV) performance. (a) Heatmaps represent LC activity from control and stressed rats during the last six correct or incorrect trials. Histograms represent LC neuronal activity expressed as z-scores normalized to the baseline for REV during the same trials collapsed. Histograms are time synchronized to reward or reward omission (red line at  $t = 0$  s). In stressed rats, LC neuronal activity was phasically increased following reward ( $*p < 0.05$ , Student's paired  $t$ -test). Phasic LC activation was not elicited by reward omission in stressed rats. It also did not occur in control rats in response to reward or reward omission. (b) Heatmaps represent the percentage of phasically activated LC neurons in response to reward (correct) or reward omission (incorrect) during REV. Heatmaps show neuronal activity from a representative control and stressed rat ( $n = 9$  cells, each rat), during REV trials (T1–T6, last six correct or the preceding six incorrect trials). Plots are time locked at reward or reward omission (white line at 0 s). For each correct and incorrect trial, burst activity was measured every 50 ms bin and expressed as the percentage of neurons that fired in each bin. Neurons from stressed rats exhibit more phasic discharge after reward ( $p < 0.05$ , Student's paired  $t$ -test), whereas controls do not ( $p = 0.7$ ).



social stress did not impair performance in any component of the task and, unexpectedly, enhanced IDS and REV performance. Notably, excessive activation of the LC–NE system has been implicated in stress-induced cognitive impairments (Birnbaum *et al*, 1999; Jett and Morilak, 2013). However, this does not occur in rats with repeated social stress history (Chajale *et al*, 2013 and the present study). Rather, LC discharge rates are inhibited compared with controls. This effect is evident for several days after the final stress exposure and it is due to an engagement of endogenous opioid afferents to the LC and downregulation of CRF receptors (Chajale *et al*, 2013). This relative inhibition of LC discharge was reproduced in the current study as indicated by the mean LC discharge rates recorded in home cages before testing in the AST. Stress-induced regulation of the LC system by opioids can account for the enhancement of IDS performance and the decreased number of perseverative errors during REV, because in humans acute morphine administration had similar positive effects on IDS and REV (Quednow *et al*, 2008). In line with the model of competing systems (Graybeal *et al*, 2011) the lower LC activity in stressed rats may facilitate a shift toward systems that favor IDS and REV. Importantly, other studies demonstrated that chronic resident–intruder stress does not impair and, in some cases, improves performance in memory tasks (Buwalda *et al*, 2005). Together, the results underscore the likelihood that different stressors will have unique cognitive consequences because they differentially affect the underlying circuits.

### Repeated Social Stress Changes the Relationship between LC Activity and Behavior

Previous studies of LC neuronal activity in monkeys and rats during behavior directed by discrete cues in operant procedures indicated that LC neurons are task responsive and not reward responsive, being phasically activated following conditioned stimuli predicting reward and preceding reward (Bouret and Sara, 2004; Clayton *et al*, 2004). Although initially LC neurons are activated by unpredicted reward, this response habituates and shifts toward task-related responses when the outcome becomes predictable. The shift from reward- to task-related activation may facilitate the development of conditioned reinforcement (Schultz and Dickinson, 2000). The task used in the present study differs from the operant procedures in requiring discrimination between cues that are simultaneously and continuously present, rather than being discretely presented so that they would not be expected to elicit a phasic response. These settings may better represent conditions under which human decisions are made because stimuli that provide information about behavioral response outcomes are unlikely to be briefly presented stimuli. Regardless of the procedural differences, the current findings in control rats are consistent with previous studies in suggesting that LC neurons are task responsive at least for correct trials, because for this group LC activity reliably increased after the decision and before the reward period. Previous studies suggested that task-related LC activity facilitates the behavior (Clayton *et al*, 2004). Consistent with this, LC neurons of stressed rats were not

task responsive and these rats took longer to dig and obtain reward.

Social stress history rendered LC neurons reward responsive during IDS and REV, and impaired habituation of LC activation by expected reward and a shift to task-related LC activation. The ability of stress to promote reward-related LC activation is consistent with an increase in reward salience and in agreement with human studies showing that stress (including psychosocial stress) increases reward salience (Lighthall *et al*, 2013; Mather and Lighthall, 2012; Petzold *et al*, 2010). Interestingly, evidence from our laboratory and others suggests that a history of stress also increases the salience of noxious stimuli based on LC responses (see below). The finding that reward-related LC activation does not habituate as reward becomes more predictable suggests that neural encoding of prediction is impaired. Neuronal encoding of prediction error, the difference between the predicted and actual occurrence of an outcome, is a basis for learning (Schultz and Dickinson, 2000). LC neurons adapt to predicted reward by shifting responses from the reward to the task (Bouret and Sara, 2004; Clayton *et al*, 2004). The findings that reward-related LC activation did not habituate or transfer to task-related activation suggests that learning at the level of these neurons was maintained in premature state in which salience of the outcome, but not the task, is high. This could explain the decreased perseveration during REV in stressed rats. LC neurons were tonically inhibited by reward omission for both groups in all task phases. This inhibition, in concert with reward-related LC activation in stressed rats, would be expected to augment learning. Notably, the effects of stress were apparent during IDS and REV but not EDS, suggesting that alternate circuits are engaged during EDS that regulate LC activity with different consequences, perhaps allowing for task-related LC activation to occur.

### Potential Cellular Mechanisms Underlying Social Stress-Induced LC Plasticity

Increased reward-related LC activity seen in the present study may be mechanistically related to enhanced foot-shock-evoked LC activity that has been reported with social stress and chronic cold stress (Bingham *et al*, 2011; Finlay *et al*, 1997). Importantly, this is also seen with the single prolonged stress model of PTSD, underscoring the potential role of this effect in symptoms of PTSD (George *et al*, 2013). Enhanced responses of LC neurons to salient stimuli could result from increased glutamatergic function in the LC, because excitatory amino acid afferents are major mediators of LC activation by discrete stimuli (Ennis and Aston-Jones, 1988). Alternatively, changes in endogenous opioid regulation of the LC may underlie these effects. Although opiates generally inhibit spontaneous LC discharge, in moderate doses they increase the signal-to-noise ratio of LC sensory responses, an effect that would favor enhanced responses to salient stimuli (Valentino and Foote, 1988).

### Clinical Implications

Social stress-induced enhancement of LC responses to reward may be advantageous to learning processes. However, these effects may also underlie some of the symptoms

of stress-related psychiatric disorders. For example, accentuated activation of the LC-NE system by rewarding stimuli such as food or drugs of abuse would be predicted to increase the arousal response to those stimuli and could underlie the role of stress in obesity and/or substance abuse. The effect of social stress to increase LC responses to reward taken with its ability to induce a cellular state of opioid dependence (Chajjale *et al*, 2013) converge to make subjects with a history of social stress particularly vulnerable to opiate abuse. Finally, the LC is poised to mediate the arousal component of emotional responses through its connections with the central amygdalar nucleus. As exaggerated emotional arousal in response to behavioral outcomes is characteristic of PTSD, the present results provide a mechanism by which repeated social stress contributes to this disease.

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