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Acute stress, but not corticosterone, facilitates acquisition of paired associates learning in rats using touchscreen-equipped operant conditioning chambers

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ABSTRACT

Acute stress influences learning and memory in humans and rodents, enhancing performance in some tasks while impairing it in others. Typically, subjects preferentially employ striatal-mediated stimulus-response strategies in spatial memory tasks following stress, making use of fewer hippocampal-based strategies which may be more cognitively demanding. Previous research demonstrated that the acquisition of rodent paired associates learning (PAL) relies primarily on the striatum, while task performance after extensive training is impaired by hippocampal disruption. Therefore, we sought to explore whether the acquisition of PAL, an operant conditioning task involving spatial stimuli, could be enhanced by acute stress. Male Long-Evans rats were trained to a predefined criterion in PAL and then subjected to either a single session of restraint stress (30 min) or injection of corticosterone (CORT; 3 mg/kg). Subsequent task performance was monitored for one week. We found that rats subjected to restraint stress, but not those rats injected with CORT, performed with higher accuracy and efficiency, when compared to untreated controls. These results suggest that while acute stress enhances the acquisition of PAL, CORT alone does not. This dissociation may be due to differences between these treatments and their ability to produce sufficient catecholamine release in the amygdala, a requirement for stress effects on memory.

1. Introduction

Stress is pervasive in society and is increasingly recognized as a cause of psychiatric and physical illness [1,2]. Acutely stressful experiences affect cognition, effects that may be relevant to brain disorders such as addiction, anxiety, and post-traumatic stress disorder (PTSD; reviewed by [3]). Impairments in spatial memory consolidation and recall are often found in people with these disorders [3]. Whereas chronic stress generally impairs aspects of hippocampal (HPC) dependent spatial memory in both humans (reviewed by Burgess et al. [4]) and rodents (reviewed by [5]), the effects of acute stress are not as consistent [6,7]. Although spatial memory recall is often similarly impaired following both acute and chronic stress [7,8], consolidation may be enhanced or impaired depending on factors such as the timing, arousal, or intensity of the stress [7,9].

The interaction between acute stress and spatial memory is influenced by the nature of the memory task used in assessment (see [10]). In rodent behaviour studies, acute stress promotes a shift from more cognitively demanding HPC-dependent strategies toward simpler, more procedural, stimulus-response (S-R) based strategies, which rely on the dorsal striatum (DSTR; [10–14]). These differences suggest a distinct mechanism by which stress hormones, primarily cortisol in humans and corticosterone (CORT) in rodents, interact with limbic and cortical structures essential for memory including the HPC, prefrontal cortex (PFC), DSTR, and amygdala [15–17].

Recent research has sought to improve the concordance between human studies and those which use animal models in stress and other fields. One method by which this has occurred is through use of analogous behavioural paradigms in both humans and rodents. One such task which has demonstrated similarities in cognition across species is paired associates learning (PAL; [18,40,19]). In humans, PAL is used clinically to detect mild cognitive impairment associated with HPC-mediated deficits in spatial associative memory in conditions such as Alzheimer's disease and schizophrenia [20,38]. A rodent version of PAL was recently developed in which visuospatial memory is assessed based on the ability to learn object-in-place associations [18]. In contrast to the human version, which is conducted in one session, the rodent version occurs over several weeks, with gradual improvement in learning

Abbreviations: PAL, Paired Associates Learning

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of the image-location pairings. Like human PAL, the rodent version is sensitive to HPC dysfunction and performance is impaired following lesions [21,22] and inactivations [22]. Furthermore, many psychoactive drugs, such as amphetamine, known to affect HPC-dependent spatial memory in humans impair retrieval and PAL performance when administered systemically or infused directly in the HPC [22–26].

However, while task performance in well-trained animals is impaired by manipulations of the HPC, acquisition of PAL is largely unaffected by pre-acquisition HPC lesions in mice [21,22] or by HPC catecholamine depletion in rats [27]. In contrast to later task performance, PAL acquisition may involve separate memory systems as lesions of the DSTR prevent PAL acquisition entirely [21].

To the best of our knowledge, no previous studies have explored the effects of stress on rodent PAL. Therefore, we first sought to determine the effect of acute stress on acquisition of this task. Previous evidence from rats and mice suggests a prominent role of DSTR-mediated memory in PAL acquisition, and we therefore hypothesized that both acute restraint stress (ARS) and CORT would facilitate this process. This was based on previous research suggesting that stress promotes preferential use of DSTR-mediated strategies rather than HPC-mediated strategies. Naïve adult male Long Evans rats were trained daily in 1 h sessions of PAL until reaching a predefined criterion early in task acquisition. The day immediately following, they were subjected to ARS (30 min), CORT (3.0 mg/kg), vehicle, or no manipulation and trained daily on PAL for an additional week.

2. Methods

2.1. Subjects

Adult male Long Evans rats (n = 58) were used for the ARS (n = 13), control (n = 13), CORT (n = 16), and vehicle groups (n = 16) (Charles River Laboratories, Kingston, NY, USA). Upon arrival at the facility, animals were pair housed and left undisturbed for 1 week with food and water *ad libitum* (Purina Rat Chow). Following facility acclimatization, animals were maintained at 90% of free feeding weight and singly housed to ensure the appropriate amount of food was consumed by each rat in the home cage after behavioural testing. Animals were housed in ventilated plastic home cages in a temperature and humidity-controlled vivarium with water available *ad libitum* except during testing. A 12:12 h lighting cycle was used with lights on at 7:00 a.m.. Animals were given environmental enrichment in their home cage in the form of a plastic tube throughout the experiment. Experiments were conducted from November 2016 to February 2017 for control and ARS animals, and from May 2017 to August 2017 for CORT and Vehicle animals. To control for normal circadian CORT rhythms, animals were trained at the same time daily. All experiments were conducted in accordance with the standards of the Canadian Council on Animal Care and the University of Saskatchewan Animal Research Ethics Board.

2.2. Training apparatus

Eight touchscreen-equipped operant conditioning chambers (Lafayette Instruments, Lafayette, IN, USA) were used for PAL (Fig. 1). Each chamber was contained within its own sound-attenuating box with a fan to provide background noise and air circulation. A live video feed of animal activity was maintained through a camera mounted within the box above the operant chamber. The chamber dimensions and layout were identical to those used previously (see Ref. [24]). A removable mask, interchangeable for different behavioural tasks, rested on the touchscreen and obscured the screen entirely except for areas where stimuli are presented, and response selection occurs. In PAL, the mask had three equally-sized rectangular response windows, arranged evenly across the mask. The windows are located above a spring-loaded response shelf that animals were required to press down to access the screen and make a selection.

2.3. Touchscreen habituation and pretraining

Habituation, pretraining, and training were conducted according to instructions and protocols established by Lafayette, and previous experiments conducted in our lab [24,25]. Animals advanced through training stages based on their individual performance and ability to meet intermediate criteria. Pretraining and training sessions occurred once daily, 6 days a week.

Animals were handled for at least 5 days before touchscreen habituation began. On the first day of habituation rats were brought from the vivarium to the touchscreen room and left undisturbed in their home cage for 1 h. They were given 5 reward pellets (Dustless Precision Pellets, 45 mg, Rodent Purified Diet; BioServ, NJ, USA) at the beginning of the habituation period. During this period, all equipment was on and the lights were dimmed to replicate the conditions used when training and testing. For all subsequent training days rats were left undisturbed for 30 min following transport to the touchscreen room.

Pretraining consisted of various intermediate and progressive steps to encourage rats to approach and nose-poke the display. It began with two 30 min chamber habituation sessions in which animals were left undisturbed in the chambers and given 5 reward pellets in the food port. Criterion was reached if all pellets were consumed within 30 min. Rats then began initial touch training in which one of the response windows was illuminated pseudorandomly. The window was illuminated for 30 s, or until touched. Three reward pellets were delivered if the rat correctly touched the illuminated window during this period and one pellet was delivered if the illuminated window was not touched. A 20 s intertrial period followed each trial. Criterion for initial touch was completion of 100 trials in 1 h. Must touch training was administered similarly, with animals receiving 1 reward pellet for correct touches only. The criterion for must touch training was 100 trials in 1 h. Must initiate training required the rat to nose-poke in the food port to initiate a trial before commencing as in the previous stage. Criterion for the must initiate phase was 100 trials in 1 h. The final stage of pretraining was the punish incorrect stage where rats were required to initiate each trial by nose-poking the food port as done previously, followed by selection of the pseudorandomly illuminated window. Correct touches to the illuminated window were rewarded with 1 food pellet, while incorrect touches were punished with illumination of the house lights and 5 s time out followed by a correction trial. During correction trials, the stimuli were repeatedly presented until a correct touch was made, triggering a food reward. A correct touch on a correction trial was recorded as a completed selection trial and followed by initiation of a new trial. The criterion for punish incorrect was 100 trials in 1 h, with greater than 80% correct, with accuracy calculated for the initial stimuli presentation only.

Rodent PAL requires the animal to differentiate between two different stimuli presented simultaneously in 2 of the 3 response windows pseudorandomly (Fig. 1; Fig. 2). Each stimulus (negative images of a flower, airplane, and spider) was correct only when paired with its respective location. The flower was always correct in the left position, the airplane in the centre position, and the spider in the right position. Correct responses were rewarded and punished in the manner previously described for punish incorrect training.

The current experiment included some adjustments to previous PAL experiments conducted in our lab. In Lins et al. [24], criteria of 100 trials, 80% accuracy for two consecutive days, and 90 trials, 80% accuracy, for 3 consecutive days were used for the punish incorrect stage and PAL task, respectively. In the present experiment, we lowered the criterions to 100 trials, 80% accuracy for one day, for punish incorrect, and to 65 selection trials, with 65% accuracy for one day in PAL. These criteria were selected based on pilot data (unpublished) in order to reduce the possibility of ceiling effects. Correction trials were not included in the count of selection trials completed and accuracy was calculated for the initial presentation of a stimulus pair only.

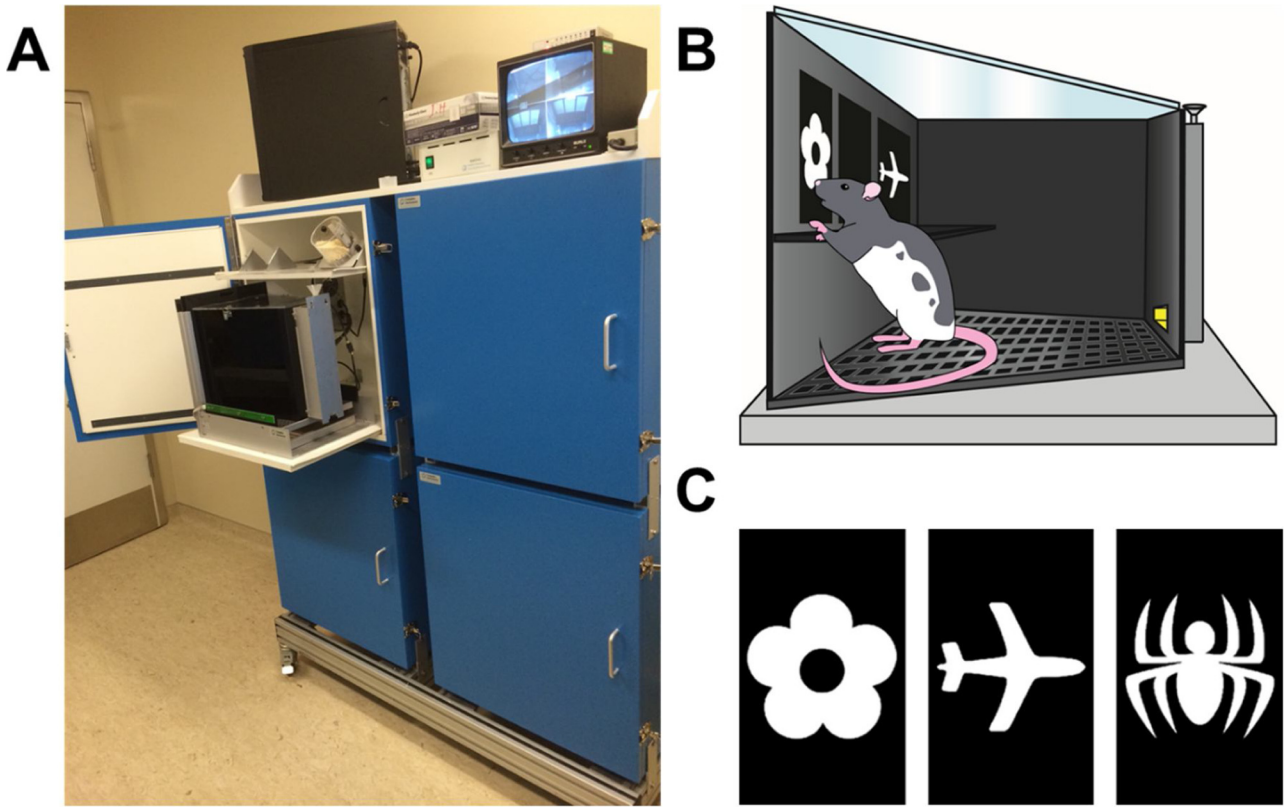

Fig. 1. Touchscreen Equipment. [A] The touchscreen equipped operant conditioning chambers are housed in sound-attenuating boxes. In addition to the touchscreen, each box has a direct camera feed, and contains an independent pellet dispenser, light, and air circulation fan. [B] The drawing represents a cross-sectional view of the touchscreen chamber. On the left side, the 3-windowed mask used of PAL obscures all the touchscreen except the active areas. A response shelf ensures the rat must stand and actively make a choice during each pairing. The rat is depicted making a correct decision by selecting the flower in the given pairing, which would be followed by delivery of a food pellet in the yellow food port on the right. [C] Each of the 3 images used for PAL shown in their respective correct positions. Each trial consists of one image in its correct position and a different image in an incorrect position (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

2.4. Acute stress procedure

Rats were randomly assigned to either the ARS (n = 13) or control (n = 13) groups, and the experimenter was blind to each rat's assigned group until treatment day. ARS animals were immobilized in a Plexiglas restraint tube (544-RR, Fisher Scientific, Ottawa, ON, Canada) in a brightly lit, novel room for 30 min. This procedure was carried out in lieu of the 30 min acclimatization period animals previously experienced. Following ARS, animals were returned to their home cage, transferred to the touch screen room, and immediately started on PAL.

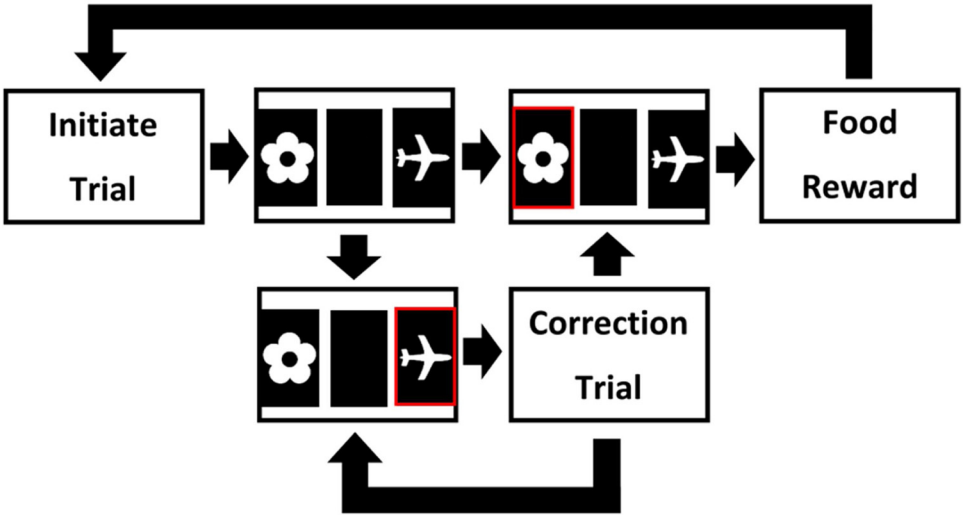

Fig. 2. PAL Full Task Schematic. A schematic representation of trials in PAL. The first trial begins with illumination of the food port and free delivery of a sucrose pellet, prompting the rat to nose poke. A nose poke initiates the trial and two different stimuli are displayed in the three response windows pseudorandomly with the third window remaining blank. One image is paired to its correct location, in this instance the flower, while the other image is not paired with its correct location, in this instance the airplane. The system then waits for the animal to decide between the two stimuli. A correct screen touch, the flower, is recorded as a completed selection trial and will result in the food reward followed by a 20 s intertrial period, at the end of which the food port will illuminate and the animal can nose poke to begin a new trial. An incorrect screen touch, the airplane, will not yield a food reward, will cause the house lights to illuminate for 5 s, and will also begin a correction trial. The correction trial consists of the same stimulus pairing and is repeated until the correct selection is made, which will yield a food reward and be counted as a selection trial. Accuracy is calculated for the initial presentation of a trial only, subsequent correction trials do not affect accuracy. The total trial measurement consists of the total number of selection and correction trials. Figure reprinted with authors permission [24].

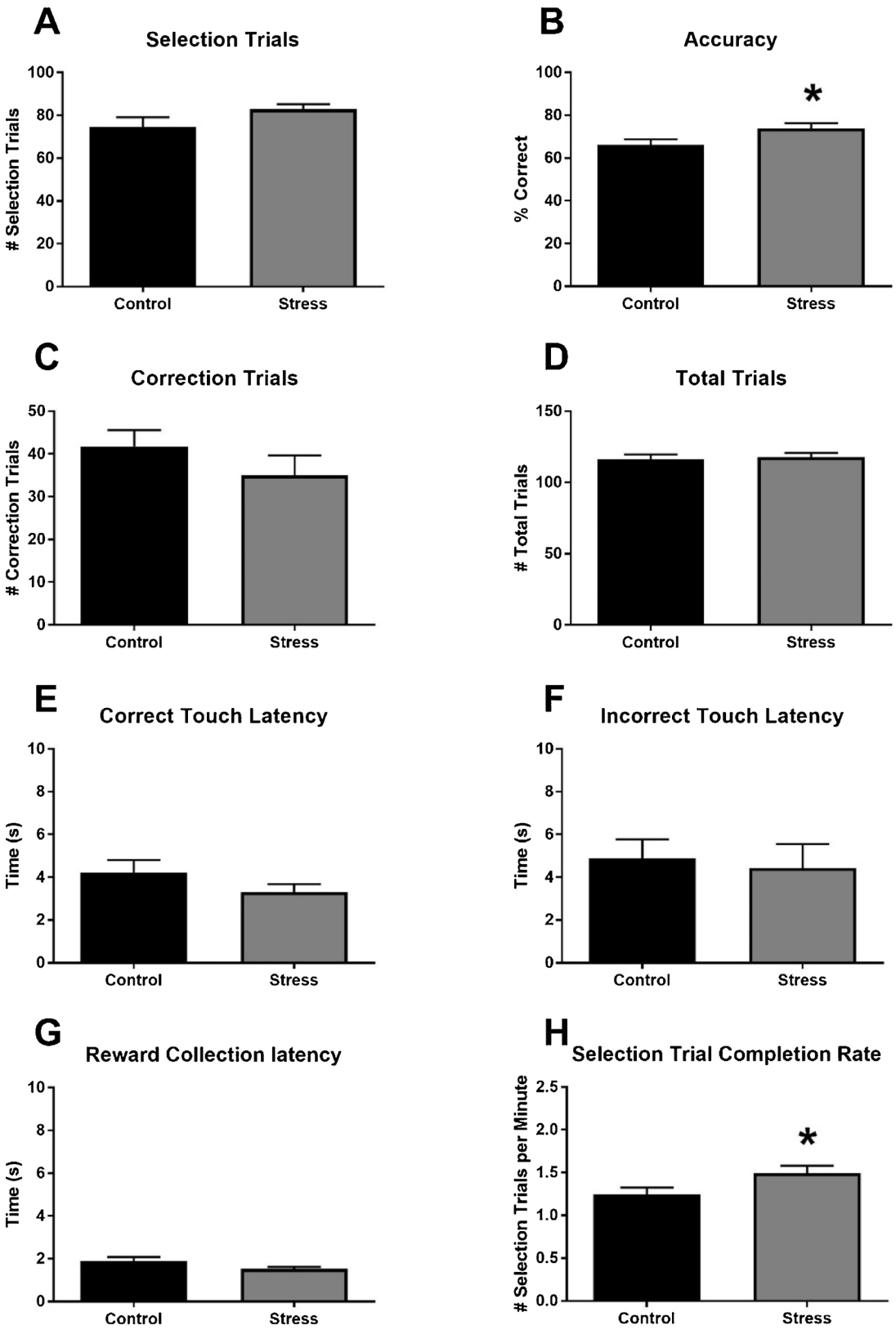

Fig. 3. Effects of acute restraint stress (ARS) on Paired Associates Learning performance on the day of stress. [A] There was no significant difference between treatment groups on the number of selection trials completed. [B] ARS animals had greater accuracy compared to controls following stress. [C–D] There was no effect of stress on the number of correction trials, or the number of total trials completed. [E–G] There was no significant difference in performance on any measure of latency. [H] ARS animals performed selection trials at a faster rate than controls following stress. * indicates pairwise comparison significant at $p < 0.05$.

PAL was initiated less than 1 min after the end of ARS. Rats exposed to ARS consistently displayed overt signs of stress including high levels of defecation, urination, and piloerection. Control animals received no ARS, were not moved to the novel room, and instead acclimatized in the touchscreen room following transport as usual.

2.5. Corticosterone treatment

Rats were randomly assigned to CORT (n = 16) or vehicle (n = 16) groups, the experimenter was blind to each rat's assigned group until treatment day. Animals were trained in PAL using the same method as above and were given either a single CORT (3.0 mg/kg) or vehicle (vegetable oil) injection (1 ml/kg; s.c.). Both CORT and vehicle solutions were prepared fresh daily and shielded from light. This procedure and dose was determined based on previous work showing CORT levels produced following ARS and the CORT injection were similar [28] and that this dose was sufficient to enhance DSTR-dependent memory [16]. Animals were injected in a novel room, with the lights dimmed to match the touchscreen room. Rats were returned to their home cage following injection and moved to the touchscreen room where they were given 30 min to acclimatize before training.

2.6. Data analysis

All data were automatically collected to prevent experimenter bias and are presented as group means ± SEM. Figures and analysis used GraphPad Prism version 7.0 (GraphPad Software, San Diego, USA). Comparisons were considered statistically significant at $\alpha \leq 0.05$. Behavioural assessment included eight factors of PAL performance: the number of selection trials performed (correct selection of appropriate stimulus on initial presentation, or following a correction trial), the number of correction trials performed, the total number of trials of all types performed (selection trials + correction trials), session accuracy (% correct responses based on initial presentation only, correction trial responses are not included), and 3 measures of task latency (correct touch latency: the time from stimulus presentation to a correct screen touch, incorrect touch latency: the time from stimulus presentation to an incorrect screen touch, and reward collection latency: the time from a correct screen touch to reward collection). Finally, as a measure of overall task efficiency, a selection trial completion rate was calculated by dividing the number of selection trials completed in a given session by the total time of the session in minutes.

Data were analyzed using independent samples *t*-Test (Treatment) and through two-way mixed model repeated measures ANOVA (Treatment by Time); post-hoc comparisons were made using a Bonferroni correction where appropriate. Data are plotted in blocks of 2 training days to improve clarity. Using this method, there were six blocks for which complete data sets for all treatments and animals were available. Blocks 1 and 2 consist entirely of pre-treatment data, with block 2 containing the day animals reached criterion. Block 3 consists of the treatment day and following session. Blocks 4–6 contain the remaining post-treatment sessions.

3. Results

After pretraining, animals completed PAL sessions until reaching a threshold of at least 65 selection trials completed with 65% accuracy in 60 min. Treatment was then delivered prior to the next session. One animal from the ARS group and one animal from the CORT group failed to acquire the task and were excluded from analysis. Final group sizes were: control (n = 13), ARS (n = 12), CORT (n = 15), and vehicle (n = 16). The minimum number of sessions required to reach criterion in PAL was 4, and the maximum was 18, with an average of 9.8 (SEM = 0.41) sessions for all groups. There was no significant difference in the number of days required to reach criterion for any group. In addition, all groups had similar performance in the session preceding treatment, and no significant differences on any performance measure were found (statistics not shown, all $p > 0.05$).

3.1. Effects of ARS on PAL acquisition on the day of stress

In experiment 1, animals received either 30 min of ARS in lieu of acclimatization or training as normal for the control condition on treatment day (Fig. 3A–H). There was no significant difference between control (74.46 ± 4.64 trials) and ARS (82.83 ± 2.41 trials) groups in selection trial completion on the day of ARS ($t(23) = 1.56$, $p = 0.13$). On the day of stress, the ARS group performed with significantly higher accuracy (73.87 ± 2.48%) compared to the control group (66.13 ± 2.65%; $t(23) = 2.12$, $p = 0.045$). There was no difference in the number of correction trials performed between controls (41.69 ± 3.87 correction trials) and ARS animals (34.92 ± 4.75 correction trials; $t(23) = 1.11$, $p = 0.28$). There was no difference in the total number of trials performed for controls (116.20 ± 3.50 total trials) or ARS (117.80 ± 2.97 total trials) animals ($t(23) = 0.35$, $p = 0.73$). Correct touch latency was not significantly different between control (4.21 ± 0.60 s) and ARS (3.30 ± 0.38 s) groups ($t(23) = 1.26$, $p = 0.22$). Incorrect touch latency was not significantly different between control (4.87 ± 0.90 s) and ARS (4.43 ± 1.13 s) groups ($t(23) = 0.31$, $p = 0.76$). Reward collection latency was not statistically different between control (1.89 ± 0.19 s) and ARS (1.53 s ± 0.09 s) groups ($t(23) = 1.68$), $p = 0.11$). The ARS group had a significantly higher selection trial completion rate (1.49 ± 0.09 trials per minute) compared to the control group (1.25 ± 0.08 trials per minute; $t(23) = 2.16$, $p = 0.042$).

3.2. ARS persistently improves PAL acquisition

Following ARS, acquisition curves were analyzed for any persisting effects of treatment. There was a total of 12 daily sessions for which data was available for all animals. To improve clarity, data were plotted in 6 blocks of 2 sessions. Blocks 1 and 2 consist entirely of pre-treatment data, with block 2 containing the day animals reached criterion. Block 3 consists of the treatment day and following session. Blocks 4–6, contained the remaining post-treatment sessions (Fig. 4A–H).

For selection trials, there were significant main effects of both Treatment ($F(5,115) = 7.94$, $p = 0.010$) and Time ($F(5,115) = 49.63$, $p < 0.001$). Both control and ARS animals improved over the course of the experiment explaining the significant main effect of Time. *Post hoc* testing showed that rats subjected to ARS performed significantly more selection trials in block 4 (88.46 ± 3.72) compared to controls (77.58 ± 3.72, $p = 0.024$). For accuracy there was a significant interaction between Treatment x Time ($F(5,115) = 3.55$, $p = 0.005$). *Post hoc* testing determined that ARS animals performed with significantly higher accuracy (77.95 ± 3.18) in block 4 compared to controls (69.19 ± 3.18, $p = 0.040$). There was a significant interaction between Treatment x Time for the number of correction trials performed ($F(5, 115) = 3.08$, $p = 0.012$), although *post hoc* tests showed no significant pairwise comparisons (statistics not shown). Both groups improved over the course of the study on total trial completion, explaining a significant main effect of Time ($F(5,115) = 3.73$, $p = 0.004$), however no group differences were identified (statistics not shown). Latency to make correct and incorrect responses decreased over the course of the study explaining the significant main effects of Time found for both correct touch latency ($F(5,115) = 3.77$, $p = 0.003$) and incorrect touch latency ($F(5,115) = 3.22$, $p = 0.009$). Latency to collect food rewards was consistent across the experiment and treatment groups (statistics not shown). Selection trial completion rate increased for both groups, explaining the significant main effect of Time ($F(5,115) = 52.29$, $p < 0.001$). There was also a significant main effect of Treatment on selection trial completion rate ($F(5, 115) = 7.84$, $p = 0.010$). *Post hoc* testing showed that ARS animals performed with a significantly faster rate during block 3 (1.55, ± 0.10 trials per minute), 4 (1.67 ± 0.10

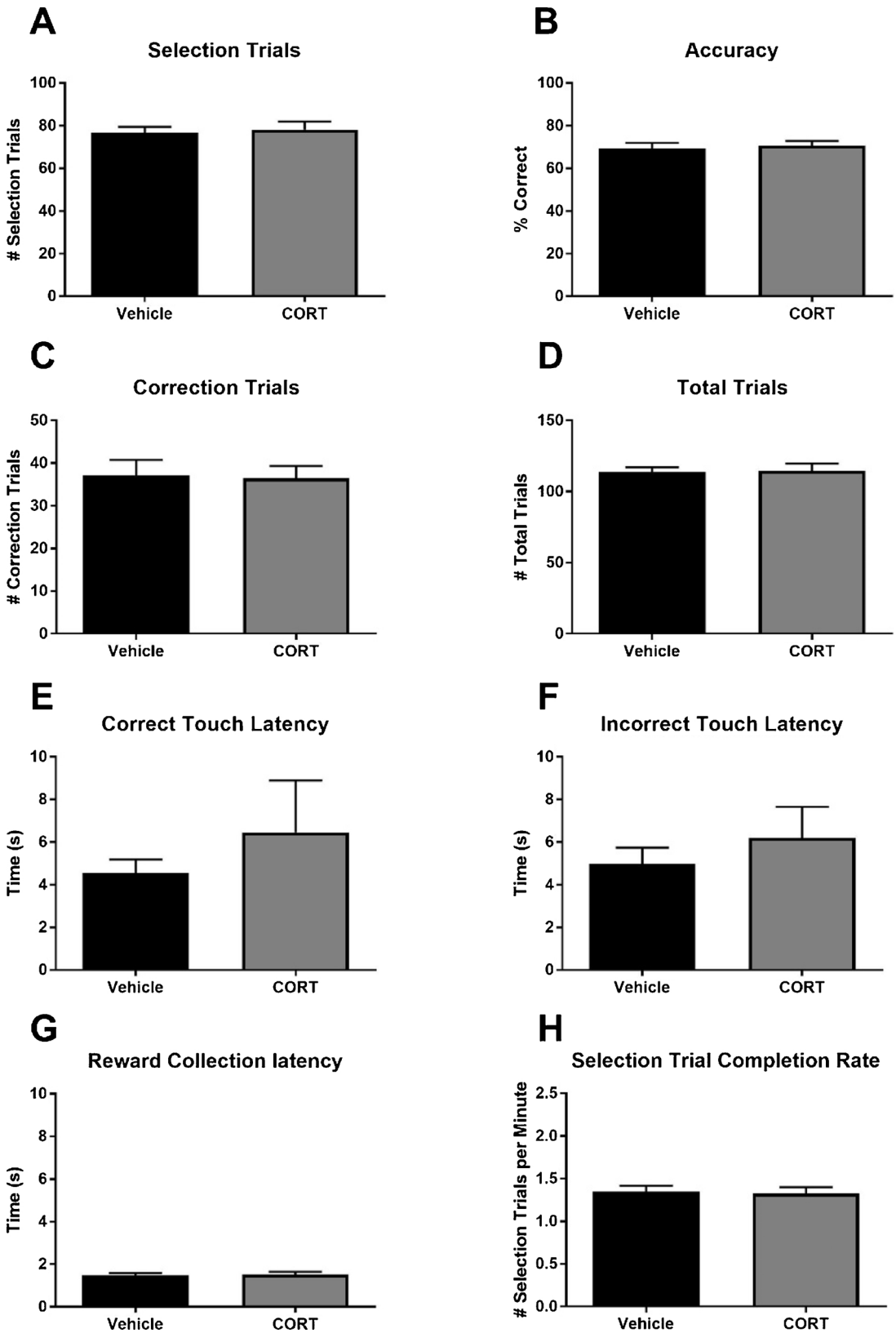

Fig. 4. Effects of corticosterone treatment on Paired Associates Learning performance on the day of injection. [A–D] There was no significant difference between treatment groups on the number of selection trials completed, task accuracy, correction trials completed, or the number of total trials completed. [E–F] Although it appeared the CORT group responded more slowly when making both correct and incorrect decisions compared to vehicle treated animals, this effect was not significant. [G–H] There was no significant difference in reward collection latency or selection trial completion rate.

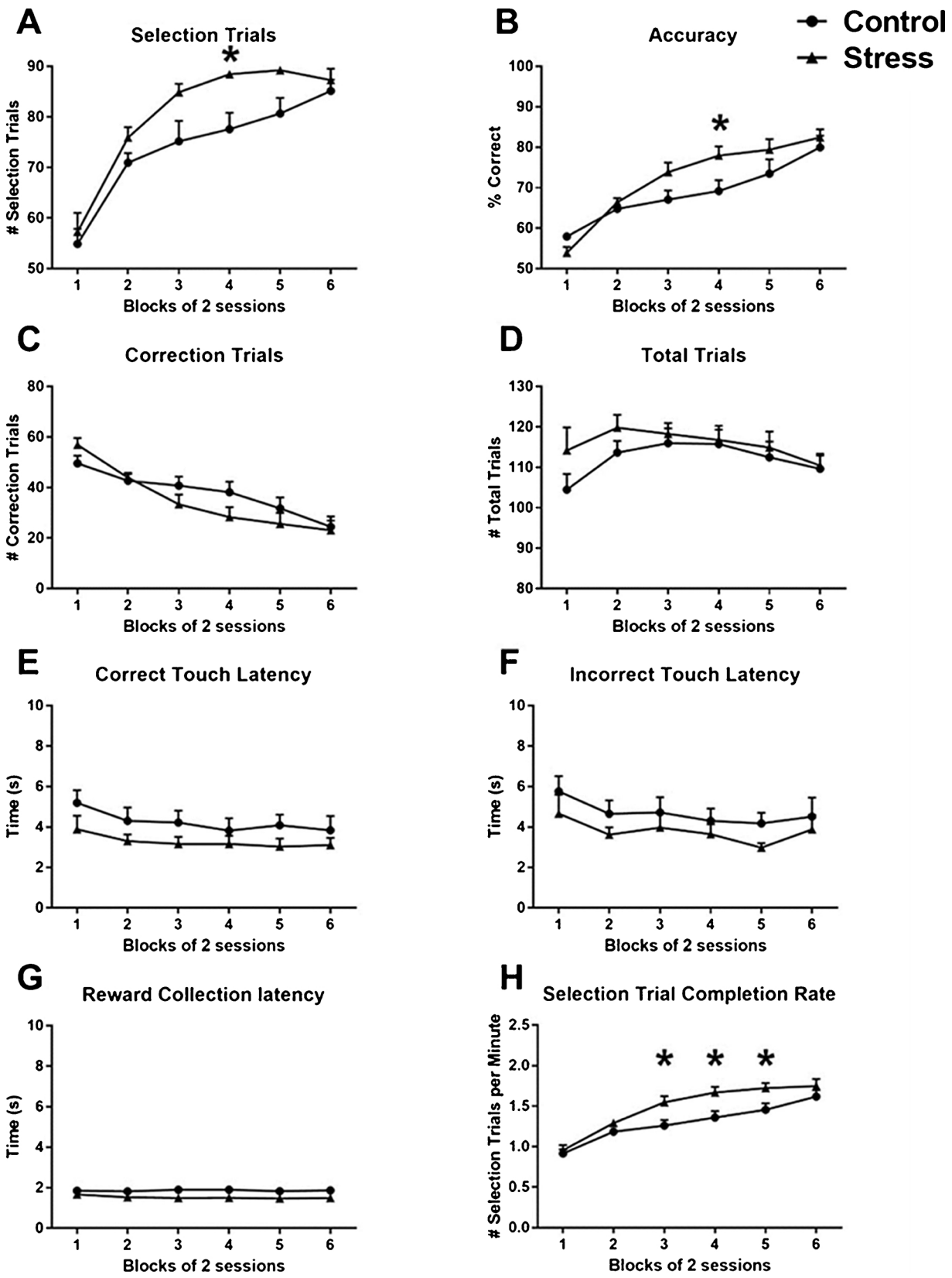

Fig. 5. Acquisition of paired associates learning before and after acute restraint stress (ARS). A total of 6 blocks were analyzed with each block consisting of 2 sessions. Blocks 1 and 2 consist entirely of pre-treatment data, block 3 contains the treatment session, and blocks 4–6, are composed of the remaining post-treatment sessions. [A] ARS animals completed more selection trials in block 4 following stress. [B] For accuracy ARS animals performed with higher accuracy compared to controls during block 4. [C] There was a significant interaction for the number of correction trials performed, but there were no significant comparisons. [D] There was a significant main effect of Time on the number of total trials completed, but there were no significant comparisons [E–F] There were significant main effects of Time for both correct and incorrect touch latency suggesting response times decreased over the course of the experiment. [G] Reward collection latency was consistent across the experiment. [H] Both groups showed significant improvement over time on the selection trial completion rate. The ARS group performed selection trials at a higher rate than controls on Blocks 3–5. See body text for further discussion of interactions. * indicates pairwise comparison significant at

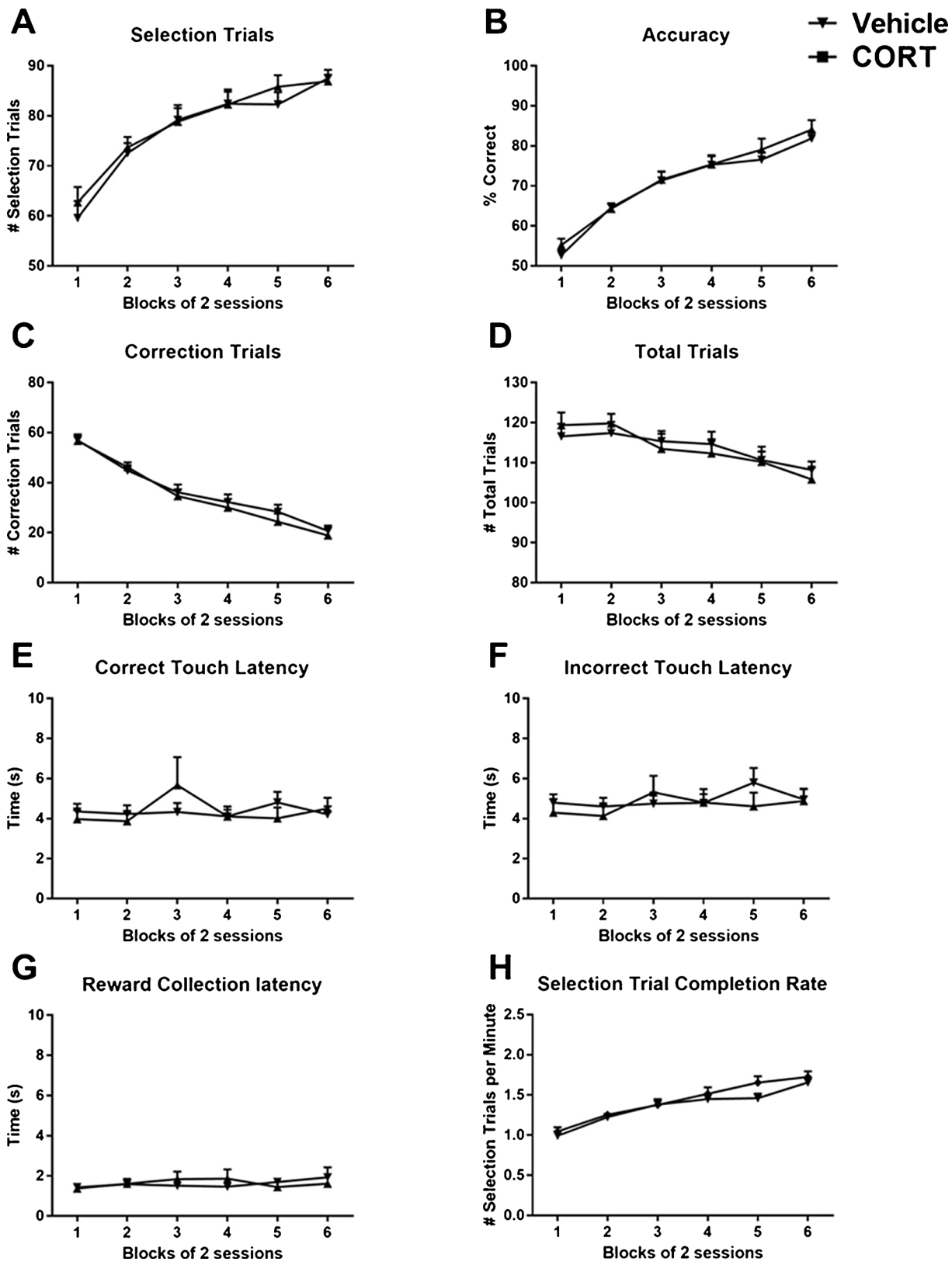

Fig. 6. Acquisition of Paired Associates Learning before and after corticosterone (CORT) injections. A total of 6 blocks were analyzed with each block consisting of 2 sessions. Blocks 1 and 2 consist entirely of pre-treatment data, block 3 contains the treatment session, and blocks 4–6, are composed of the remaining post-treatment sessions. [A–D] Both CORT and vehicle groups improved over the course of the experiment explaining the significant main effect of Time for selection trial completion, accuracy, correction trial completion, and total trial completion. [E–G] There was no improvement or decrement in any latency measure over time. [H] Both groups showed significant improvement over time on the selection trial completion rate.

trials per minute), and 5 (1.72, ± 0.10 trials per minute) than controls for blocks 3 (1.19, ± 0.10 trials per minute, $p = 0.020$), 4 (1.26 ± 0.10 trials per minute, $p = 0.011$), and 5 (1.36, ± 0.10 trials per minute, $p = 0.040$) respectively.

3.3. Effects of CORT on PAL acquisition on the day of treatment

In experiment 2, animals received either CORT (3.0 mg/kg) or vehicle on treatment day (Fig. 5A–H). Independent sample t-tests failed to identify any significant differences between CORT and vehicle-treated animals on any measure of task performance in the session immediately following treatment (statistics not shown).

3.4. No difference between CORT and vehicle treated animals during PAL acquisition

Acquisition curves were analyzed for any persisting effect of CORT on PAL acquisition (Fig. 6A–H). Performance improved for both groups over the course of the experiment explaining significant main effects of Time in the number of selection trials completed ($F(5,145) = 38.24$, $p < 0.001$), accuracy ($F(5,145) = 120.40$, $p < 0.001$), correction trials ($F(5,145) = 130.00$, $p < 0.001$), and total trials completed ($F(5,145) = 10.23$, $p < 0.001$). There was no significant effect of either Treatment or Time on correct touch latency, incorrect touch latency, or reward collection latency (statistics not shown). Both groups increased selection trial completion rate over the course of the experiment, explaining the significant main effect of Time ($F(5,145) = 52.25$, $p < 0.001$). There were no significant differences between groups on any measure assessed (statistics not shown).

4. Discussion

The results of the present study showed that a single 30 min session of ARS was sufficient to enhance the acquisition of PAL while systemic administration of CORT was not. Furthermore, this facilitation persisted for subsequent sessions while the rats were mastering the task. We believe this result is due to an enhancement of S-R learning following acute stress, consistent with previous work showing that acute stress enhances the acquisition of S-R learning in spatial memory tasks [13] and promotes behaviours which may rely on the DSTR [13,16].

We originally hypothesized that acute stress would impair PAL performance on the treatment day but enhance acquisition over the long term. Our hypothesis was based on previous work showing that acute stress generally impairs spatial memory retrieval, while enhancing consolidation [7,17,29,30]. However, the improved PAL acquisition observed following ARS is not entirely inconsistent with previous work, as some studies have shown that acute stress enhances performance in behavioral tasks including the Morris water maze [6,30], cued-water maze [13] and reversal learning ([31]; but see also [41,32]). Thus, both the immediate and persistent improvement of PAL performance following acute stress may be produced by preferential selection of less cognitively demanding learning strategies which are generally employed following stress [14,33,39]. This theory fits well with previous literature demonstrating decreases in HPC-dependent strategies coupled with increases in DSTR-mediated behavioural strategies following acute stress [10,14,33].

While we theorize the improved acquisition demonstrated here is the result of preferential enhancement of DTSR-mediated S-R strategies, we cannot exclude the possibility that HPC-mediated strategies were also enhanced by stress. In contrast to other behavioural tasks which can be used to distinguish between DSTR and HPC-based learning strategies, such as the plus maze [27,33], it's likely that neither system is exclusively involved in PAL acquisition. Both S-R and cognitive learning strategies could be used to acquire PAL: a S-R strategy where each of the six-possible pairings are memorized (e.g., if: flower left and spider right, choose flower), or where a rule is learned and applied (e.g., the flower is always, and only, correct on the left). Although research has shown that PAL acquisition relies on an intact DSTR [21] and can occur without a functional HPC [21,27], post-acquisition performance is readily impaired by HPC disruption [21,22,27]. It is reasonable to expect that pre-acquisition HPC lesion biases animals toward an S-R strategy, but this does not exclude use of HPC-dependent strategies in intact animals. Therefore, the improvement in PAL acquisition following stress may not be due to selective improvement of one strategy, rather a general enhancement to spatial learning which was not strategy specific, although future research will be required to confirm this.

Interestingly, there was no difference between CORT and vehicle treated animals. Although many studies have shown that both ARS and 3.0 mg/kg CORT produce similar effects on spatial memory [14,16] this is not always the case [16,42,43]). Fear-induced CORT elevation impairs HPC-dependent spatial memory, leaving HPC-independent memory intact, while CORT elevation alone does not have an effect [34]. Furthermore, fear-conditioning has been shown to enhance DSTR-dependent memory [17]. Therefore, we propose that the improved task acquisition seen in the ARS group may be attributable to stress-induced elevations in CORT levels as well as concomitant increases in catecholamine release and emotional arousal. Enhanced S-R memory following stress has previously been shown to rely on noradrenaline release in the DSTR [16]. However, as we did not measure regional activities or hormone concentrations, this theory cannot be confirmed from this experiment alone.

Although we believe these experiments demonstrate a difference between the effects of ARS and CORT in the acquisition of PAL, there are limitations which must be considered when interpreting these results. For example, as the dose of CORT used (3.0 mg/kg) was moderate and circulating CORT was not measured following the treatments, it is possible that stress caused by vehicle injection was sufficient to generate a similar stress response, obscuring effects of CORT. Furthermore, previous research has demonstrated that exposure to a novel environment produces a measurable stress response [35,36] and both vehicle and CORT-treated rats were taken to a novel room for injection. As the ARS and CORT experiments were conducted in serial and the different groups were trained months apart, cohort effects may hinder comparison. Ceiling effects may also limit interpretation of these results. PAL acquisition occurs over many weeks and generally concludes when an animal completes 90 selection trials per session with over 80% accuracy for 3 sessions. In our study we sought a time point for the manipulations in which animals were performing more selection trials than correction trials, consistently above chance (> 50% accuracy), while also maximizing potential for improvement. However, as the session concludes once the rat completes 90 selection trials, regardless of the number of total trials, this measure is limited by innate task constraints, while performance limits other parameters. To adjust for this, we calculated the selection trial completion rate which is the total number of selection trials completed, and thus food rewards received, in a minute. As accuracy, correction trials, and latencies influence this measure, we propose this rate represents a general measure of task efficiency. A final possible explanation for this difference may relate to the novelty of the behavioural task and susceptibility to stress. Previous work has shown that when animals are habituated to a treatment environment, they exhibit less pronounced mnemonic effects following stress [37]. As PAL pretraining and acquisition occurs over many weeks, it is reasonable to expect these animals were well habituated to the training environment, and thus may exhibit little arousal. Therefore, the differences between the effects of ARS and CORT should be interpreted cautiously.

5. Conclusion

These experiments demonstrate that ARS, but not CORT, is sufficient to enhance the acquisition of PAL in rats. Acute stress has been shown to enhance learning of S-R associations and promote behaviours

which are mediated through the dorsal striatum, we theorize this provides a possible explanation. As we found no effect of CORT alone on task acquisition, we suggest that this facilitation must rely on interaction with other systems relevant to stress and memory, particularly those involving catecholamines and the amygdala. Further research is required to directly assess this theory. Together, this work fits well with previous literature suggesting an important role for the DSTR in the acquisition of PAL, and with studies that demonstrate that ARS can enhance learning in tasks requiring spatial memory. The interaction between stress and memory in PAL is important as previous work supports continued use of touchscreen-based methods in preclinical and translational research.

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