ACUTE STRESS, BUT NOT CORTICOSTERONE, FACILITATES ACQUISITION OF PAIRED ASSOCIATES LEARNING ASSESSED IN RATS USING TOUCHSCREEN-EQUIPPED OPERANT CONDITIONING CHAMBERS

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ABSTRACT

Acute stress is well known to influence learning and memory tasks in humans and rodents, enhancing performance in some instances while impairing it in others. Across species, subjects preferentially employ striatal mediated stimulus-response strategies in spatial memory tasks following stress, making use of fewer hippocampal based strategies which are thought to be more cognitively demanding. Previous research has demonstrated that the acquisition of rodent paired associates learning (PAL) relies primarily on the striatum, while later task performance can be impaired through hippocampal disruption. Therefore, we sought to explore whether the acquisition of this task could be enhanced by acute stress. Male Long-Evans rats were trained to a predefined criterion in PAL and were subjected to either a single session of restraint stress (30 min) or injection of corticosterone (CORT; 3 mg/kg). Daily performance was then monitored for one week. We found that only the animals subjected to restraint stress performed with higher accuracy and task efficiency, when compared to untreated controls. These results suggest that while acute stress enhances the acquisition of PAL, CORT alone does not. This may be due to differences which have been identified between these treatments and their ability to produce sufficient catecholamine release in the amygdala, a requirement for stress effects on memory. However, as the effect of restraint stress was moderate and not significantly improved over CORT, these results should be interpreted with caution until these findings are replicated.

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As part of my training, I've spent several decades attempting to beat all emotions from my body so that I may enlighten my mind and focus on the three C's of scientific success: Critical thinking, Cynicism, and Sarcasm. However, writing an acknowledgments section veered dangerously close to the expression of feelings, therefore I initially considered several possible alternatives to fill the void, yet still save face: a recipe for a mediocre, slightly dry banana bread; a 400-page *Game of Thrones* / *Lord of the Rings* crossover fanfiction; a list of random times and arbitrary GPS locations; and lastly, the words "All work and no play make Andy a dull boy" repeated in size four font to fill the page. However, after trying each, I felt only shame for ignoring those who have been so supportive, encouraging, and instrumental to this work. Therefore, in attempt to reconcile these two sides of my psyche, I've opted to use the "sandwich method". There are three steps to the sandwich method: 1. Start with a long a winded explanation to feed my ego. 2. Follow it up with a section where I'm genuine, thanking everyone for their support and guidance, and well… let's face it, putting up with me in general. 3. End with a snappy quip or a little joke to reaffirm that I'm an iconoclast. Step 1 complete.

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Phew, made it. Could be a record? But with steps 1 & 2 done, I just need to wrap it up with a jo

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LIST OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid
AMY	Amygdala
ANOVA	Analysis of variance
ARS	Acute restraint stress
BBB	Blood-brain barrier
BLA	Basolateral amygdala
CA1	Cornu ammonis 1
CNS	Central nervous system
CORT	Corticosterone
CREB	cAMP response element-binding protein
CRH	Corticotropin-releasing hormone
DHPC	Dorsal hippocampus
DSTR	Dorsal striatum
GRE	Glucocorticoid response element
GRs	Glucocorticoid receptors
HHPS	Hypothalamic-hypophyseal portal system
HPA Axis	Hypothalamic-pituitary-adrenal axis
HPC	Hippocampus
K+	Potassium ion
LTP	Long-term potentiation
LTD	Long-term depression
mEPSC	Miniature excitatory postsynaptic currents
MD	Mean difference
MRs	Mineralocorticoid receptors
MWM	Morris water maze
NA	Noradrenaline

NMDA	N-Methyl-D-Aspartate
PAL	Paired associates learning
PFC	Prefrontal cortex
PNS	Peripheral nervous system
PTSD	Post-traumatic stress disorder
PVN	Paraventricular nucleus
R-O	Response-outcome
s.c.	Subcutaneous injection
SEM	Standard error of the mean
SNARE	SNAP receptor
S-R	Stimulus-response

1. INTRODUCTION

1.1 Stress Physiology

Stress is pervasive in society and is increasingly recognized as a cause of psychiatric and physical illness (Dimsdale 2008; Schneiderman et al., 2008). However, while stress may become pathological if prolonged or particularly intense, the stress response plays integral roles in the maintenance of essential physiological processes and mediates many functions necessary for adaptation and survival. In humans, the effects of stress and largely realized through interactions produced by many stress related hormones such cortisol, aldosterone, adrenaline, noradrenaline (NA), corticotropin-releasing hormone (CRH), vasopressin, and many others (McEwen, 2007). These stress hormones interact with most body systems allowing for a diverse response. For example, stress elevates oxygenation and glucose metabolism in preparation for increased metabolic demand (McEwen, 2007), increases arousal and alertness (Hermans et al., 2011), generates the rapid release of stress hormones found during the waking response (Clow et al., 2010), and can promote memory formation and retrieval (de Quervain, 2016). Together, these different attributes allow one to act in a self-preserving manner, attend to relevant information and threats, adapt as needed, and respond appropriately in complex environments.

Activation of the hypothalamic-pituitary-adrenal axis (HPA axis) is a consequence of experiencing acute stress. The HPA axis is composed of two central nervous system (CNS) structures, the hypothalamus and pituitary glands, as well as the adrenal glands located on the superior aspect of the kidneys (McEwen, 2007). At rest, negative feedback of the HPA axis maintains an appropriate level of circulating stress hormones (McEwen, 2007). However, these levels are not static, and fluctuate normally in a consistent circadian rhythm leading to higher levels of stress hormones following waking, for both humans (Saper et al., 2005) and rodents

(Liston et al., 2013). During normal homeostatic function, or in response to a specific stressor, activation of the HPA axis begins with the limbic system, primarily composed of the hippocampus (HPC), amygdala (AMY), cingulate gyrus, mammillary bodies, and septal nuclei, among other structures (Smith & Vale, 2006). In response to sensory input, cognitive appraisal, changing levels of circulating stress hormones, or other stressful stimuli, the limbic system initiates a stress response through activation of the hypothalamus (Smith & Vale, 2006). The most prominent pathway of HPA axis activation stems from glutamatergic neurons originating in the AMY which travel through the amygdalofugal pathway and stria terminalis, subsequently activating neurons in the periventricular nucleus (PVN) of the hypothalamus (Smith & Vale, 2006). The PVN then triggers release of CRH from the median eminence into the hypothalamic-hypophyseal portal system (HHPS), where it travels through vascular tissue to the anterior lobe of the pituitary gland (Smith & Vale, 2006). In the anterior pituitary, CRH initiates the synthesis and release of adrenocorticotropic hormone (ACTH) into the bloodstream that subsequently stimulates steroidogenesis and release of corticosteroids, catecholamines, and androgenic steroids from the adrenal glands (Smith & Vale, 2006). ACTH promotes the biosynthesis of cortisol and aldosterone from cholesterol in the adrenal cortex, while both sympathetic stimulation and an increase in corticosteroids increase release of the catecholamines adrenaline and NA from the adrenal medulla (McEwen, 2007).

From there, stress hormones exert their effects systemically, affecting virtually all body cells through either direct or indirect mechanisms, increasing activity associated with the sympathetic branch of the autonomic nervous system (Schneiderman et al., 2008). This leads to increases in cardiovascular activity, heart rate, and blood pressure, as well as increasing blood glucose levels through increased catabolic activity and lipolysis (McEwen, 2007). However, while these

metabolic effects help to ensure a long-term energy supply, stress hormones acutely decrease pancreatic insulin secretion and reduce uptake of glucose by non-neuronal extrahepatic cells (Steptoe et al., 2007). The net effect of these functions is to ensure metabolic reserves are maintained for essential tissues. However, other systems may be deprived by chronically elevated stress hormones, leading to fatigue, damage, apoptosis, and muscle wasting if such a state persists (McEwen, 2007). In addition to the metabolic effects, release of stress hormones also coincides with decreasing levels of many molecules relevant to immune response, inhibiting formation of prostaglandins, interleukins, cyclooxygenases, and other proinflammatory molecules, as part of an overall suppression of this system (Steptoe et al., 2007).

Increased metabolic activity and a general suppression of the immune system following stress are essential adaptive responses, but become hazardous if such states are maintained. The body's cells become resistant to the effects of stress hormones, resulting in greater insulin secretion and hyperglycemia, increases in basal heart rate and blood pressure, and an elevated immune response during periods of rest and normal physiological function (McEwen, 2007). Thus, it is essential that the HPA axis not only responds rapidly to stress, but also that it is capable of dynamically downregulating the same responses when demands change. This is accomplished through a negative feedback loop in which elevated levels of lipid soluble stress hormones pass through the blood-brain barrier (BBB), stimulating inhibitory mechanisms in limbic areas of the brain (Smith & Vale, 2007). The interplay between the relative activation, and subsequent suppression of the HPA axis allows the body to both maintain a consistent daily rhythm and adapt to increased levels of stress and environmental demand.

1.2 Glucocorticoid and Mineralocorticoid Receptors in the Brain

While activation of the HPA axis is systemic, most responses and downstream functions are accomplished directly through activation of mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs) by their respective mineralocorticoids and glucocorticoids. In both humans and rodents, the most abundant mineralocorticoid is aldosterone, whereas the predominant glucocorticoid is cortisol in humans, and corticosterone (CORT) in rodents (McEwen, 2007). Both aldosterone and CORT are released from the adrenal glands following stress (McEwen, 2007). Glucocorticoid receptors are ubiquitous, present in nearly every cell of the body, and are expressed in most brain regions (Morimoto et a., 1996). In contrast, although expressed in peripheral tissues including the heart, colon, and kidneys, MRs are generally restricted to the limbic areas and prefrontal cortex (PFC) within the CNS (Herman 1989; Vogel et al., 2016). For both GRs and MRs, there are several isoforms of each receptor and, in neurons, both may be found as membrane bound variants which appear to mediate rapid responses, or as nuclear forms which play a greater role in the regulation of gene transcription and long-term effects (Popoli et al., 2012).

For both membrane and nuclear receptors, the effects of GRs and MRs are pleiotropic, which can induce, supress, or otherwise alter expression of more than 10% of the human genome (Oakley & Cidlowski, 2013). However, there appears to be substantial differences between these two receptor classes. Activation of nuclear receptors, both GRs and MRs, is thought to play a prominent role in transcriptional regulation with targets determined based on the specific isoforms involved (Oakley & Cidlowski, 2013). The nuclear receptors form homodimers and heterodimers in response to corticosteroids, which are lipophilic and can easily cross the cell membrane (de Quervain et al., 2009; Gomez-Sanchez & Gomez-Sanchez, 2014). These dimers

translocate to the nucleus, where they bind directly to DNA regions such as the glucocorticoid response element (GRE), regulating transcription and expression of several genes important to adaptation and survival (de Quervain et al., 2009; Oakley & Cidlowski, 2013). In contrast, activation of membrane receptors acts through secondary messengers, such as heat shock proteins, which broadly affect transcription, or through receptor internalization and effects similar to that of the nuclear isoforms (Oakley & Cidlowski, 2013; McEwen et al., 2016).

In general, GRs have lower affinity for all corticosteroids, mineralocorticoids and glucocorticoids, and are more selective for endogenous glucocorticoids and the commonly used exogenous agonist dexamethasone (Di & Tasker, 2008). This is in comparison to mineralocorticoid receptors that bind cortisol and CORT with higher affinity than GRs; but are also activated in response to aldosterone (Rogerson et al., 2004). During normal physiological function, these differences in binding affinity lead to a comparatively higher level of MR saturation in tissues that express both receptor types (Popoli et al., 2012). This appears to relate to somewhat distinct functional roles, with GRs regulating many of the responsive elements following stress, such as metabolic and immune effects, while MRs play a greater role in maintaining general HPA homeostasis, steroid receptor concentration, cell survival, as well as regulating water and ion transport (Popoli et al., 2012). However, although the differences in distribution and function of MRs and GRs allow for adaptation and regulation of stress responses on both the short and long term, significant overlap and interaction suggests these systems are not isolated competing processes but rather they operate in parallel to one another.

Although a major function of these receptors is gene regulation and more long-term effects in the body and brain, the membrane variants are thought to be highly involved in the immediate stress response, particularly in the CNS (Vogel et al., 2016). Acting through second messenger

systems, neuronal membrane GRs stimulate memory consolidation (Barsegyan et al., 2010; Roozendaal et al., 2010), inhibit working memory (Barsegyan et al., 2010), increase endocannabinoid signalling (Campolongo et al., 2009), and decrease vasopressin release (Wang et al., 1995). Following acute stress, activation of membrane GR receptors produces rapid insertion of AMPA subunits in postsynaptic hippocampal neurons (Conboy & Sandi, 2010). This process that may rely on concurrent catecholamine release (Zhou et al., 2012; de Quervain et al., 2016). Through another mechanism, both footshock stress and CORT enhance glutamate transmission by increasing availability of SNARE protein complexes which increases glutamate release in presynaptic neurons (Musazzi et al., 2010). Furthermore, there is evidence that membrane GRs may also influence transcription by increasing CREB activation (Chen et al., 2012) and through promotion of histone acetylation and epigenetic mechanisms (de Quervain et al., 2016).

Similarly, activation of the membrane MR produces rapid effects following stress. Studies show that it increases excitation by stimulating neuronal glutamate release, while simultaneously inhibiting K^+ currents (Olijslagers et al., 2008), and by increasing AMPA receptor availability (Groc et al., 2008). Furthermore, CA1 neurons respond with a higher frequency of miniature excitatory postsynaptic currents (mEPSCs) following membrane MR activation (Karst et al., 2005). However, in comparison, much less research has focused directly on the role of the membrane MR following stress, as until recently it was thought to be largely saturated at resting physiological levels (Vogel et al., 2016). The recent demonstration that the membrane MR displays a much lower binding affinity for corticosteroids when compared to the nuclear variant (Karst et al., 2005) has led to an increase in research implicating it in many cognitive processes, such as learning and memory, following stress (Vogel et al., 2016).

1.3 Neurobiology of Learning and Memory Following Acute Stress

While the fundamental mnemonic effects of stress in the brain are often attributed to changes at the cellular and subcellular levels, broader changes are also extremely pronounced. Stress can alter spine density, neurogenesis, and neuronal complexity in the AMY, HPC, and PFC (Uysal et al., 2012; Kirby et al., 2013; McEwen et al., 2016) and has been shown to increase dendritic branching in striatum (Taylor et al., 2014). Functionally, stress can alter many synaptic properties as well, inducing long-lasting potentiation in PFC pyramidal neurons (Yuen et al., 2011), disrupting subicular potentiation and plasticity (MacDougall & Howland, 2013), and by increasing HPC long-term depression (LTD; Wong et al., 2007). The apparent contradictory nature of these effects following stress appears to originate from differences in the timing, intensity, and duration of the stress in relation to the phenomena being measured. As discussed above, the different affinities of various steroid receptors allow for a versatile response to changing concentrations of stress hormones. In a study exploring memory consolidation in rats, the enhancing effects of a GR agonist were shown to follow a traditional inverted U-shaped dose-response relationship, where moderate doses enhanced consolidation, while lower and higher doses had no effect (Roozendaal et al., 1998). Similar effects have been demonstrated in a number of studies conducted in both humans and rodents (de Quervain et al., 2017).

However, the concentration of steroid hormones alone is not sufficient to explain why stress has a particular effect on memory processes in some instances, but has a different effect in others. Studies have demonstrated that the mnemonic effects of stress depend on coincident release of NA in the basolateral amygdala (BLA). In a study exploring the role that emotional arousal plays in modulating the effects of CORT on object recognition in rats, Okuda et al., (2004) found that performance was enhanced only when animals were naïve to the experimental

procedure, and that there was no change in retention memory when animals were well habituated. A follow-up to this work showed that the effect of CORT on learning and memory in object recognition required concurrent noradrenergic activation in the BLA, and that these effects could be blocked entirely through administration of the ß-adrenoceptor antagonist propranolol (Roozendaal et al., 2004). A similar relationship has been identified in human studies, which have also identified an essential role for the membrane MR in this response (Schwabe et al., 2012; Vogel et al., 2015). Together, the results of these studies strongly suggest that emotional arousal is required for acute stress to have an effect on memory in many species (Hermans et al., 2011).

Stress hormones have been proposed to effect two important aspects of spatial memory: consolidation and retrieval. However, there are differences in the effects of stress on these two processes. Consolidation, the molecular changes that occur as part of long-term memory storage, can be enhanced, impaired, or entirely unaffected, by stress. These differences are due to interactions between stress and factors such as sex (Conrad et al., 2004), emotional arousal (Roozendaal et al., 2004), timing (Wiegert et al., 2006), and the underlying memory systems involved (Schwabe et al., 2010). Thus, the effects of acute stress on spatial memory consolidation are difficult to generalize without consideration being given toward specific experimental variables. As a result, consolidation will be further discussed below within the context of the behavioural studies.

In contrast to the facilitation occasionally seen during consolidation, acute stress generally impairs spatial memory retrieval. Rodent studies have found that CORT administration impairs retrieval in the Morris water maze (MWM; de Quervain et al., 1998), and Y-maze (Wright et al., 2006), and that both CORT and vehicle injections were sufficient to impair performance in the

radial arm maze (Atsak et al., 2016). Furthermore, timing was important for the impairment of recall as both vehicle and CORT given 30 min before testing impaired recall, but had no effect if administered without a delay (de Quervain et al., 1998; Atsak et al., 2016). The time delay required for recall impairment fits with the delay in stress hormone elevation, 10 – 20 min (Droste et al., 2008), and suggests it is linked to the behavioural effect (Atsak et al., 2016). As neither endogenous CORT elevation, nor systemic administration of CORT elevates neuronal hormone levels immediately, the time requirement fits well with literature suggesting recall impairment selectively relies on non-genomic effects mediated through membrane GRs (Chauveau et al., 2010).

1.4 Effects of Acute Stress on Hippocampal and Striatal Mediated Memory

A single stressful event can produce both acute and lasting effects, which are thought to arise from interactions between stress hormones and mnemonic processes. This has implications relevant to psychiatric disorders such as post-traumatic stress disorder (PTSD; de Quervain et al., 2017). However, these effects are not always consistent, and stress may impair or enhance behavioural performance in a task-specific manner. Particularly interesting are the effects that have been generated in spatial memory research, in which stress appears to promote a shift from more cognitive demanding strategies toward simpler habitual behaviours. Thus, it appears that the effects of stress on memory are not only related to the duration, context, and intensity of the stress, but may also be heavily influenced by the memory system employed (Schwabe & Wolf, 2012).

Converging research suggests that these conflicting results may reflect different strategies employed in each task, and differences in the relative involvement of the dorsal striatum (DSTR) and HPC following acute stress (Vogel et al., 2016; Goldfarb & Phelps, 2017). Briefly, this

theory suggests that following stress, behaviours switch from more complex and cognitively demanding HPC-dependent strategies to simpler, less cognitively demanding, habitual strategies that rely more heavily on the DSTR (Goldfarb & Phelps, 2017). Behaviours that make use of spatial associations and place cues (e.g. orientation based off a familiar landmark) are thought to reflect HPC based 'cognitive' memory. In contrast, S-R behaviours (e.g. red-stop, green-go), are thought to be mediated through the DSTR both during (Featherstone & McDonald, 2005a) and after acquisition (Featherstone & McDonald, 2005b).

To assess these systems independently, behavioural studies have made use of dual-solution tasks to assess whether a hippocampal or striatal strategy has been employed (Goldfarb & Phelps, 2017). Several rodent dual-solution tasks exist, such as the plus-maze (De Leonibus et al., 2011) or circular-hole board (Schwabe et al., 2010), but each generally follows a similar theme in which the subject freely acquires a task through use of spatial cues (e.g. an X on the wall) or through S-R (e.g. at the end of the maze, turn right). Following several acquisition trials, a probe trial is introduced that allows the strategy employed to be assessed. Use of spatial cues and allocentric strategies are attributed to HPC memory systems while S-R and cue dependent strategies have been attributed to striatal memory (Packard & Wingard, 2004; Goldfarb & Phelps, 2017). Furthermore, following stress, behaviours tend to favor the use S-R strategies, even if the task was acquired using a different approach (Hawley et al., 2013; Leong & Packard, 2014). In agreement with the rodent literature, similar results have been found in humans in which activity in the DSTR was positively correlated with task performance following stress, while HPC activity was positively correlated with performance in control conditions, and negatively correlated following stress (Schwabe & Wolf, 2012).

However, although strong evidence demonstrates that DSTR-mediated strategies and behaviours are preferentially deployed following stress, the relative effects on the HPC are not as clear. A recent review of the human and rodent literature described three theories to explain how these two structures may interact at a systems level following stress (Goldfarb & Phelps, 2017). The first theory suggests that acute stress enhances striatal memory while impairing HPC memory. Support for this theory has been found in many studies exploring both human and rodent behaviour (Schwabe & Wolf, 2012; Hawley et al., 2013; Leong & Packard, 2014). A second theory suggests that performance across both systems degrades, but that HPC-based strategies are more severely impaired. Support for this theory comes from research that has found recall impairments in both DSTR (Atsak et al., 2016) and HPC (Park et al., 2008) mediated tasks. Lastly, a third theory would be that HPC-behaviours are not impaired by stress, rather striatal circuits are instead preferentially selected for and enhanced. While comparatively little work has investigated this possibility, post-encoding stress that enhances HPC memory in rodents (Wingard & Packer, 2008) has been shown to also enhance striatal memory (Goodman et al., 2015). In this case, an animal may substitute S-R behaviours for previously used place strategies although the other system was not impaired in any way. However, regardless of which theory regarding the relative involvement of the HPC and DSTR is ultimately validated, across each theory the available evidence broadly suggests an increase in S-R behaviours in spatial memory following acute stress.

1.5 Paired Associates Learning and Acute Stress in Rodent Spatial Memory

Rodent spatial memory tasks are commonly used in translational research exploring aspects of learning, memory, and stress (Bussey et al., 2012). This is partially due to a relatively high level of concordance between some human and animal studies (Talpos et al., 2009;

Nithianantharajah et al., 2012, 2015). Recently, there has been a focused effort to develop translational behavioural paradigms wherein a task may be delivered to a human participant, while an analogous, often simplified, version is used in an animal model. Development of these tasks has proceeded through both bottom up methods, where animal tasks are extrapolated to humans, and top down methods where human behavioural tasks are simplified for animal use (Bussey et al., 2012).

In acute stress research, some promising translational results have been generated through the use of water maze tasks in rodents, and a virtual water maze in humans. In rats, acute stress facilitated consolidation in the MWM, which was correlated with an increased release of trophic factors in the HPC (Uysal et al., 2013), with a similar behavioural result found in humans (van Gerven et al., 2016). Furthermore, in both humans and rodents, HPC damage impairs performance in both the traditional (Broadbent et al., 2006) and virtual water maze (Goodrich-Hunsaker et al., 2010). However, these results are not entirely consistent as acute stress promoted HPC-mediated behaviours in humans (van Gerven et al., 2016) rather than those mediated through the DSTR in animals (Atsak et al., 2016) and an MR agonist enhanced retrieval in humans (Piber et al., 2016), whereas CORT impairs retrieval in rodents (de Quervain et al., 1998; Wright et al., 2006). However, despite these differences, tasks such as these provide an initial framework by which translational behavioural studies may be conducted.

Human behavioural tasks have also been modified for use with rodents using touchscreen-equipped operant conditioning chambers. Rodent paired associates learning (PAL) is a visuospatial associative memory task recently developed to complement the human version of PAL, in which memory is assessed based on the ability to learn object-in-place associations (Talpos et al., 2009). In humans, PAL is used clinically to detect mild cognitive impairment

associated with HPC-mediated deficits in spatial memory in conditions such as Alzheimer's disease and schizophrenia (Rover et al., 2011). In contrast to the human version, which is conducted in one session, the rodent version occurs over several weeks, with gradual improvement in learning of the image-location pairings. However, like studies in humans, rodent PAL is sensitive to HPC lesions (Delotterie et al., 2015; Kim et al., 2015) and deactivations (Kim et al., 2015). Furthermore, many psychoactive drugs known to effect HPC spatial memory in humans impair retrieval and PAL performance when administered systemically or infused directly (Talpos et al., 2009; Lins et al., 2015; Kim et al., 2015; Roschlau et al., 2016).

However, although translational behavioural paradigms such as the water maze can be used to detect HPC spatial impairment in humans and rodents, PAL may have unique advantages, particularly for stress research. First, although task performance is impaired through manipulations of the HPC, acquisition of PAL is largely unaffected by pre-acquisition HPC lesions in mice, suggesting involvement of other memory systems (Delotterie et al., 2015; Kim et al., 2015). Lesion of the DSTR prevents PAL acquisition entirely (Delotterie et al., 2015). Recent studies conducted with Listar rats found that depleting the HPC of catecholamines, which are important to spatial memory learning (McNamara et al., 2014), was not sufficient to impair acquisition of PAL (Roschlau & Hauber, 2017). Furthermore, the same study found catecholamine depletion facilitated a switch from place strategies to S-R when rats were tested in the T-maze (Roschlau & Hauber, 2017). The apparent requirement of the DSTR for acquisition of PAL may reflect the relative contributions of the dorsomedial striatum and dorsolateral striatum for response-outcome (R-O) and S-R learning, respectively (Delotterie et al., 2015). If these stages of PAL performance are indeed reliant on different memory systems, then this task may allow for a dissociation between DSTR-mediated acquisition and the HPC-mediated recall.

Furthermore, PAL may offer a means to reduce variability, which has complicated research regarding the role of acute stress. PAL data collection occurs automatically and with minimal experimenter involvement, reducing potential confounds that may be particularly damaging to research involving acute stress (Lewejohann et al., 2006). Lastly, many currently used dual-solution tasks are limited to a single probe session. As PAL occurs over many weeks, both acute and lasting effects of any manipulation may be measured.

1.6 Hypothesis and Expected Results

To the best of our knowledge, no previous studies had explored the effects of stress directly on PAL. Therefore, we first sought to determine what effect acute stress might have on acquisition of this task. Previous evidence from rats and mice suggests DSTR-mediated memory is essential for PAL acquisition, and we therefore hypothesized that both 30 min acute restraint stress (ARS) and 3.0 mg/kg CORT would facilitate this process. This was based on studies that found S-R associations mediated through the DSTR to be facilitated following acute stress.

However, due to substantial evidence implicating the HPC in PAL, and studies that have found that acute stress impairs spatial memory recall, we hypothesized PAL performance may be negatively affected in the session immediately following stress. However, as previous research suggests recall impairments are due to elevated hormone at the time of testing, we expected this effect to be limited, and that an overall facilitating effect would be seen in subsequent training sessions.

2. METHODS

2.1 Subjects

Adult male Long Evans rats (n = 58) were used for 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 single housed and maintained at 90% of free feeding weight for the duration of the study. Water was available *ad libitum* except during testing. Animals were housed in ventilated plastic home cages in a temperature and humidity controlled vivarium. A 12:12-h lighting cycle was used with lights on at 7:00am. Animals were given environmental enrichment in the form of a plastic tube throughout the experiment. Experiments were conducted from September 2016 to April 2017 (Squad 1: ARS and control animals) and June 2017 to September 2017 (Squad 2: CORT and vehicle animals). Animals in Squad 1 were randomly distributed between two training cohorts, with schedules overlapping throughout this period. To account 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 paired associates learning (Figure 1). Each chamber was contained within its own sound-attenuating box and a vented fan provided background noise and air circulation. A live feed of animal activity was maintained through a camera mounted within the box above the operant chamber. The chambers themselves were trapezoidal with the

touchscreen positioned as the wall of the wide base, and a food port inset within the wall of the narrow base. The dimensions were 240 mm at the screen, 126 mm at the feeder, a depth of 332mm and a height of 300 mm. An interchangeable mask, used for different behavioural tasks, rested on the touchscreen, obscuring the screen entirely except for areas exposed by the response windows. In PAL, the mask had three equally-sized rectangular response windows, each measuring 150 mm x 60 mm, arranged evenly across the mask with the narrow edges arranged along the horizontal plane separated by 15 mm. The touchscreen windows for the PAL task sat above a spring-loaded response shelf and animals were required to stand to make a selection.

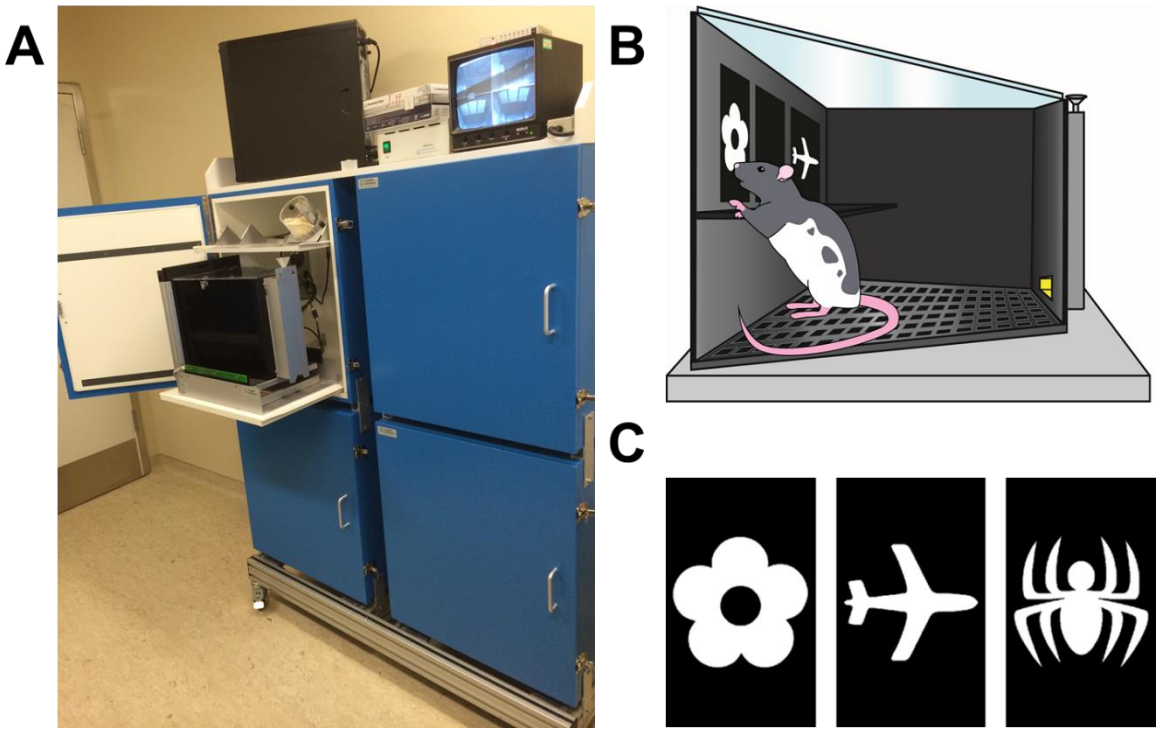


Figure 1: Touchscreen Equipment. [A] The touchscreen equipped operant conditioning chambers are housed in two blocks of four sound-attenuating boxes. In addition to the touchscreen, each box has a direct camera feed, and contains an independent pellet dispenser, light, and air circulation fan. **[B]** 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.

2.3 Habituation and Pretraining (refer to Table 1)

Habituation, pretraining, and training were conducted according to instructions and protocols established by Lafayette (see Bussey et al., 2012), and previous experiments conducted in our lab (Lins et al., 2015). Animals were free to advance through training stages based on their individual performance and ability to fulfill intermediate criteria. During pre-training stages animals were trained six days a week. During the full task animals were trained daily until experiment completion.

Animals were handled for at least 5 days before touchscreen habituation began. On the first day of habituation animals 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 start of the habituation period. During this period, all equipment was on and the lights were dimmed. For all subsequent training days rats were given an acclimatization period and left undisturbed for 30 min following transport to the touchscreen room.

Pretraining consisted of many intermediate and progressive steps. It began with 2 30-min chamber habituation sessions in which animals were left undisturbed in the operant chambers and given five reward pellets in the food port. The 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 by a white rectangle pseudorandomly. The window was illuminated for 30 s. Three reward pellets were delivered if the rat correctly touched the illuminated window during this period, whereas one pellet was delivered if the illuminated window was not touched. A 20 s intertrial period followed each trial. The 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. The must-initiate training was conducted similarly with the inclusion that the animal must nose poke the food port to initiate a trial. Criterion for the must initiate phase was 100 trials in 1 h. The final stage of pretraining was the punish incorrect stage. Rats were required to initiate each trial by nose poking the food port, which caused one of the response windows to illuminate pseudorandomly. Correct touches to the illuminated window were rewarded with one food pellet, incorrect touches were punished with a 5 s time out, illumination of the house lights for 5 s, and a correction trial. Correction trials are identical to the previous presentation and repeat until successfully completed. The criterion for punish incorrect was 100 trials in 1 hr, with greater than 80% correct. Accuracy is calculated for the initial presentation only.

Table 1: Timeline of training and treatment events.

Squad 1		Squad 2	
Week	**Stage**	**Week**	**Stage**
1	Handling and Habituation	1	Handling and Habituation
2-4	Pretraining	2-4	Pretraining
5-6	Early PAL acquisition	5-6	Early PAL acquisition
6-7	Treatment: Restraint Stress, Control	6-7	Treatment: CORT, Vehicle
7-8	Post-treatment PAL training	7-8	Post-treatment PAL training
9-10	Experiment completion	9-10	Experiment completion

2.4 Paired Associates Learning

Rodent Paired Associates Learning (PAL), requires the animal to differentiate between two different stimuli presented simultaneously in 2 of the 3 response windows (Figure 2). Each stimulus is correct only when paired with its respective location. The stimuli are negative images of a flower, airplane, and spider. The flower is always correct in the left position, the airplane in the centre position, and the spider in the right position. In total, six different configurations are possible, each consisting of a single correct image, a single incorrect image, and a blank position. Each trial is presented pseudorandomly such that no more than two successive trials have the same configuration, and each possible configuration occurs the same number of times. Each daily session of PAL continues for 1 hr or until 90 selection trials have been completed.

Correct and incorrect responses are rewarded and punished in the same manner as during the pretraining stages, with a sucrose pellet or 5 s timeout respectively. Incorrect responses result in a correction trial in which the same image pairing is presented. Correction trials repeat until the correct response is made, at which point the animal receives a food reward and a new trial may begin, however task accuracy is based solely off the initial presentation of a given trial.

Animals were trained daily until they could complete 65 selection trials in a single day, with greater than 65% accuracy on the initial presentations. In the next session after reaching criterion, animals either continued through training untreated for control animals, or were subjected to one of three treatments prior to training: 30 min ARS, 3.0 mg/kg CORT in 1.0 ml/kg vegetable oil, or 1.0 ml/kg vegetable oil alone. All treatments began 30 minutes before the daily PAL session. Following the treatment day, animals returned to daily training as normal for another 7 sessions.

The current experiment makes some adjustments to previous PAL experiments conducted in our lab. In Lins et al., (2015), criterions 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 have 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 points were based on pilot data, and aimed to reduce the possibility of ceiling effects. All training and treatments were performed by a single researcher for ARS and control experiment (Squad 1), whereas two researchers jointly conducted the CORT and vehicle experiment (Squad 2).

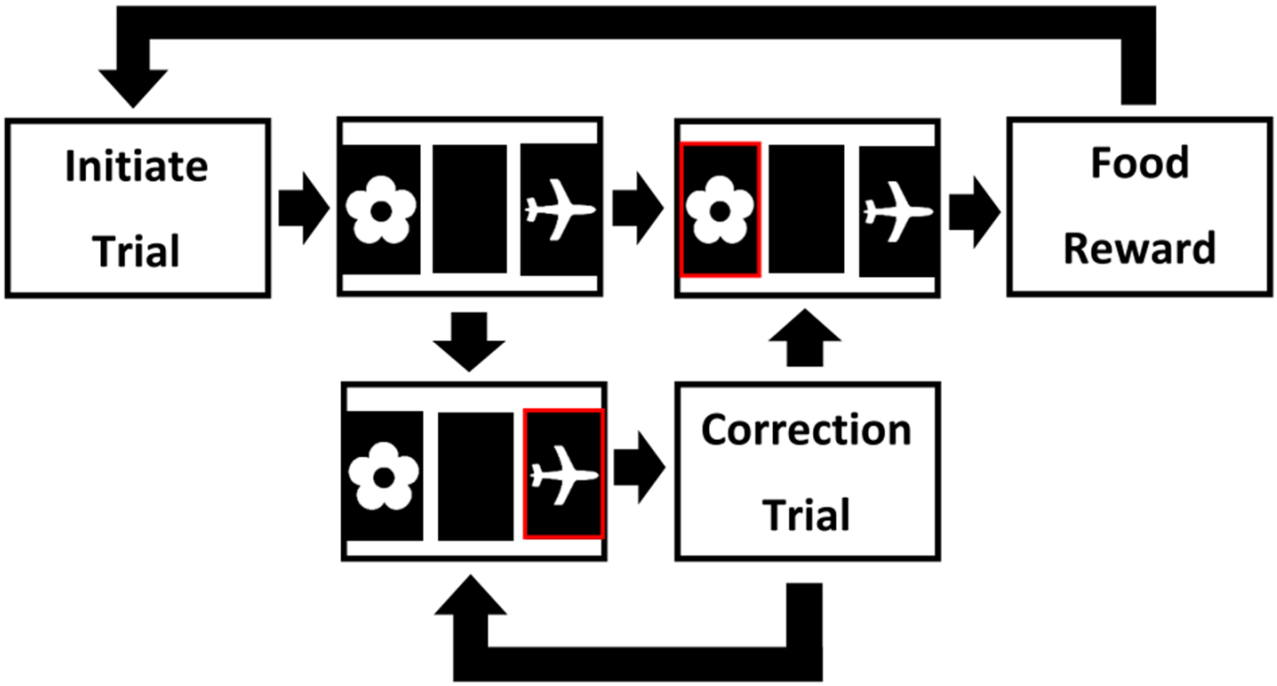


Figure 2: PAL Full Task Schematic. A schematic representation of trials in PAL. The first trial begins with illumination of the food port and free delivery of a sucrose pellet, prompting the rat to nose poke. A nose poke initiates the trial and two different stimuli are displayed in the three response 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 computed based on the initial presentation of a trial only and is unaffected by successive correction trials. The total trial measurement consists of the total number of selection and correction trials.

2.5 Acute Stress Procedure

Rats (n = 26, control = 13) were randomly assigned to either the ARS or control groups, and the experimenter was not aware of group membership for an animal 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. PAL was initiated less than one minute after the end of stress. Rats exposed to ARS consistently displayed overt signs of stress including high levels of defecation, urination, and piloerection. Control animals received no ARS, and were not moved to the novel room.

2.6 Corticosterone Procedure

Rats (n = 32; vehicle = 16) were randomly assigned to CORT or vehicle groups, one experimenter was blind until treatment day, while the other was blind for the entirety of the experiment. A single experimenter performed all injections. Animals were trained in PAL using the same method as above and were given either a single s.c. CORT or vehicle injection in lieu of ARS with a dose of either 3.0 mg/kg CORT suspended in 1 ml/kg vegetable oil, or 1 ml/kg vegetable oil alone for the vehicle treatment. Both CORT and vehicle were prepared fresh daily and shielded from light. This procedure and dose was determined based on previous work conducted in this lab (MacDougall & Howland, 2013). Animals were injected in a novel room, with the lights dimmed to match the touchscreen room. In contrast to the ARS procedure, rats in both the CORT and vehicle group displayed few overt behavioural signs of stress. They were returned to their home cage following injection and moved to the touchscreen room where they were given 30 minutes to acclimatize and returned to regular training.

2.7 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). An alpha level of $p < 0.05$ was used to determine whether comparisons were considered statistically significant. Behavioural assessment includes eight factors of PAL performance: The number of selection trials performed, the number of correction trials performed, and the total number of trials of all types performed (selection trials + correction trials). Furthermore, session accuracy (% of correct responses) was calculated based on the first presentation of stimulus only, decisions made in subsequent correction trials were not included in accuracy. Three separate measures of task latency were used: correct touch latency is the time from stimulus presentation to a correct screen touch, incorrect touch latency is the time from stimulus presentation to an incorrect screen touch, and reward collection latency is the time from a correct screen touch to reward collection. All latencies were measured through radiofrequency beams within the chamber. Furthermore, 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.

Acute analysis was performed using one-way ANOVA. Long term analysis was conducted using two-way mixed model repeated measures ANOVA, comparisons were made using Tukey, with a between factor of Treatment and a within factor of Time. Data were plotted in blocks of 2 trials to improve graphical depiction. 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, 5, and 6, contain the remaining post-treatment sessions.

3. RESULTS

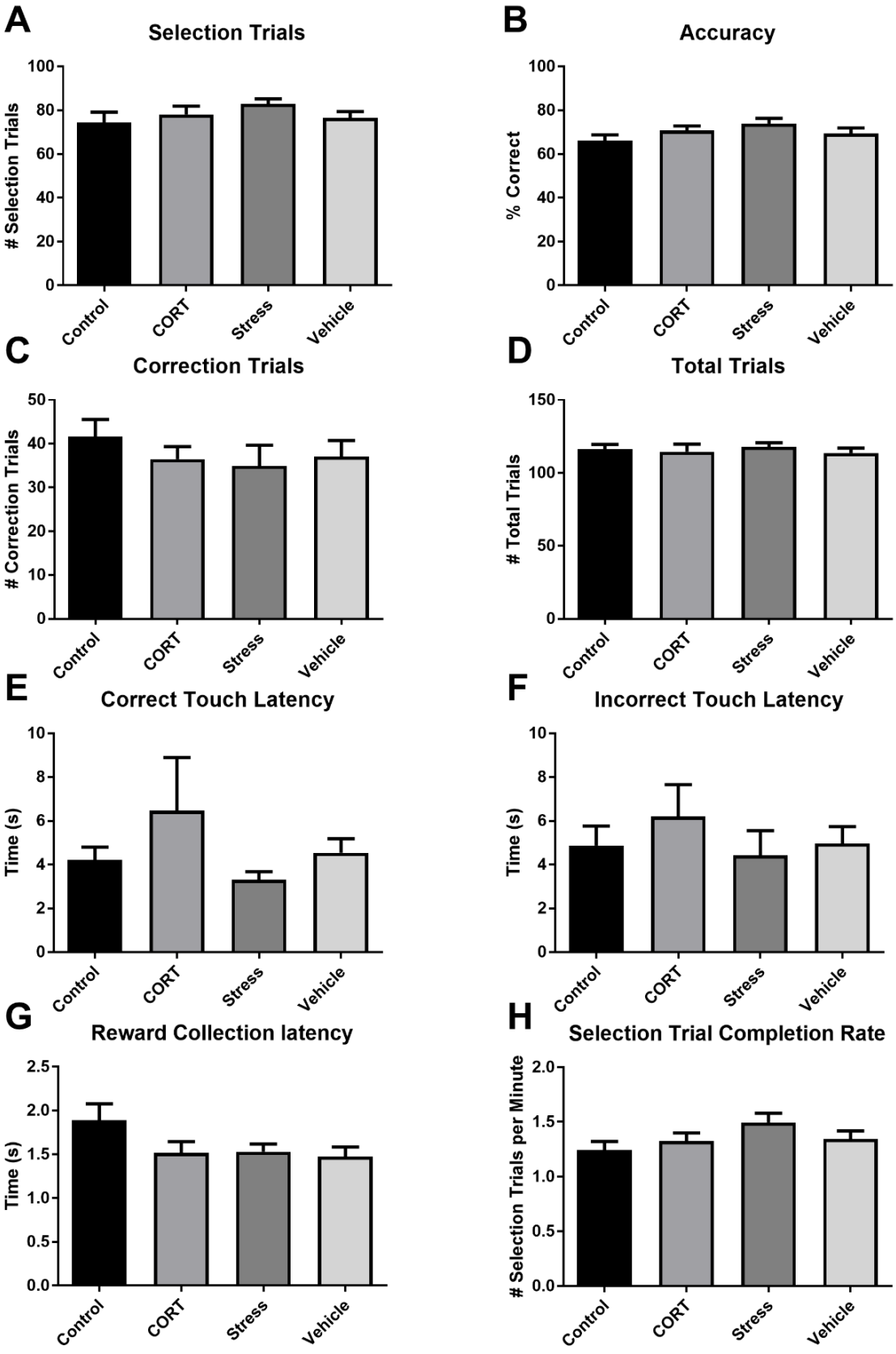
3.1 Pre-treatment PAL sessions, performance, not significantly different across groups

After completing the pretraining stages, animals completed PAL sessions until reaching a threshold of at least 65 selection trials completed with 65% accuracy in 60 min, after which treatment was delivered in the next session. One animal from the ARS group and one animal from the CORT group failed to acquire the task and were removed from the experiment entirely. 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 slight variation in the number of PAL sessions to reach criterion between groups with controls averaging 11.0 sessions (SEM = 1.1), ARS averaging 9.2 (SEM = 0.7), CORT averaging 10.1 (SEM = 0.7) and the vehicle group averaging 8.8 (SEM = 0.7). A one-way ANOVA identified no significant group difference in the mean number of PAL sessions required to reach criterion ($F(3,52) = 1.51$, $p = 0.223$).

Performance was similar across groups and there was no significant difference on any measure for the session preceding treatment (Statistics not shown, all $p > 0.05$). The average number of selection trials was 79.5 (SEM = 1.15) completed at an average rate of 1.4 per minute (SEM = 0.03) for all groups. The average number of correction trials was 38.4 (SEM = 1.24), and total trials was 117.9 (SEM = 1.40). The average accuracy before treatment was 70.9 (SEM = 0.61) percent correct. The average latency for correct decisions, incorrect decisions, and reward collection was 3.9 s (SEM = 0.23), 4.3 s (SEM = 0.26) and 1.6 s (SEM = 0.07), respectively.

3.2 No difference in treatment day performance across groups on any measure

On the treatment day, animals received either 30 min ARS (stress), a s.c. injection of 3.0 mg/kg CORT in 1 ml/kg vegetable oil (CORT), a s.c. injection of 1 ml/kg vegetable oil (vehicle), or no treatment at all (control). A one-way ANOVA showed no significant group difference in task accuracy ($F(3,52) = 1.51$, $p = 0.223$; Figure 3B). All animals performed a similar number of selection trials ($F(3,52) = 0.89$, $p = 0.452$; Figure 3A), correction trials ($F(3,52) = 0.55$, $p = 0.650$; Figure 3C), or the number of total trials completed ($F(3,52) = 0.21$, $p = 0.889$; Figure 3D). Although CORT animals had greater correct and incorrect latency, there was no significant difference in decision making time across groups for either correct touch ($F(3,52) = 0.90$, $p = 0.448$; Figure 3E) or incorrect touch latency ($F(3,52) = 0.48$, $p = 0.698$; Figure 3F). Control animals took longer to collect rewards following a correct decision, however analysis of reward collection latency revealed the effect was not significant ($F(3,52) = 2.03$, $p = 0.121$; Figure 3G). Stress animals appeared to perform with a higher selection trial completion rate following treatment, compared to controls, CORT, and vehicle treated animals, however analysis again found that this effect was not significant ($F(3,52) = 1.61$, $p = 0.197$; Figure 3H).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Selection Trials per Minute
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Stress
Vehicle

Figure 3: Treatment day effects on Paired Associates Learning [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 all other groups, this effect was not significant. **[G]** There was no significant difference in reward collection latency across groups. **[H]** Stress animals appeared to perform selection trials at a faster rate than other groups following treatment, however the effect was not statistically significant.

3.3 Stress animals perform with greater accuracy and a higher selection trial completion rate compared to controls in sessions following treatment

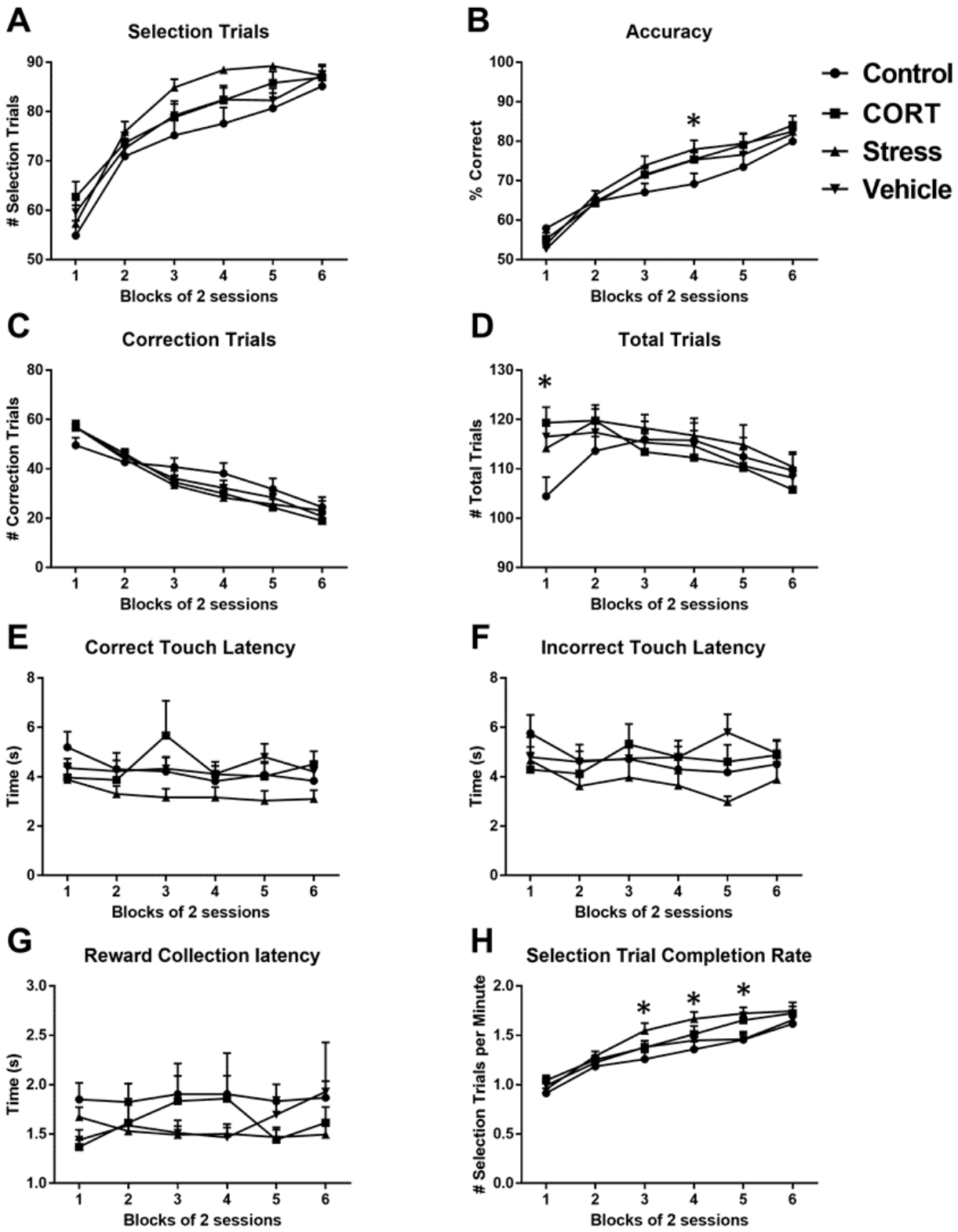
To determine whether there were any persisting effects of treatment on PAL acquisition, animals were trained for 8 sessions following initial treatment. Data was binned into 6 2-session blocks, with blocks 1 & 2 containing the pre-treatment data, block 3 containing the treatment session, and blocks 4, 5, and 6, containing the post-treatment sessions. A 2-way repeated measures ANOVA with factors of Treatment x Time was used to examine the effects on several measures of PAL acquisition.

All animals showed an increase in the number of selection trials performed over the duration of the experiment explaining the significant main effect of Time ($F(5,260) = 86.2$, $p < 0.0001$; Figure 4A). There was trend toward a difference between groups in the number of selection trials performed, however there was no significant main effect of Treatment ($F(3,52) = 2.53$, $p = 0.067$; Figure 4A). For accuracy, there was a significant interaction between Treatment x Time ($F(15,260) = 1.93$, $p = 0.021$; Figure 4B). *Post hoc* analysis found that ARS animals performed with significantly higher accuracy than control animals on block 4 (MD = 8.76, SEM = 3.11, $p = 0.026$; Figure 4B). There was a significant interaction between Treatment x Time in the number of correction trials performed ($F(15,260) = 2.23$, $p = 0.006$; Figure 4C), *post hoc* tests found no significant comparisons. There was also a significant interaction between Treatment x Time in the total number of trials performed ($F(15,260) = 2.13$, $p = 0.009$; Figure 4D). *Post hoc* testing found that both CORT (MD = 14.91, SEM = 4.66, $p = 0.083$) and vehicle (MD = 12.1, SEM = 4.59, $p = 0.044$; Figure 4D) animals performed significantly more total trials than controls in block 1, preceding any treatment.

In contrast to the overall improvement in trials and accuracy over time, latency measures did not significantly change over time (all $p > 0.05$). The ARS group consistently made correct

decisions more quickly compared to the other groups, but analysis of correct touch latency failed to identify a significant treatment effect ($F(3,52) = 1.20$ $p = 0.320$; Figure 4E) or interaction ($F(15,260) = 1.46$ $p = 0.122$). The ARS group also appeared to make incorrect decisions more quickly than other groups, however analysis failed to reveal a main treatment effect ($F(3,52) = 0.86$ $p = 0.467$; Figure 4F), although the interaction between Treatment x Time approached significance ($F(15,260) = 1.635$ $p = 0.065$). Control animals performed with higher reward collection latency across the span of the study, however there was no significant group effect ($F(3,52) = 0.82$ $p = 0.488$) or interaction ($F(15,260) = 0.756$ $p = 0.726$; Figure 4G).

All groups improved on the selection trial completion rate over the course of the experiment, explaining the significant main effect of Time ($F(5,260) = 104.2$ $p < 0.0001$; Figure 4H). Additionally, there was a main effect of Treatment ($F(3,52) = 2.81$ $p = 0.048$), but no significant interaction $F(15,260) = 1.34$ $p = 0.180$). *Post hoc* testing revealed that the ARS group performed with a higher selection trial completion rate compared to controls on blocks 3, 4, 5, and a higher rate than the vehicle group on block 5.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E
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Time (s)
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F
Incorrect Touch Latency
Time (s)
Blocks of 2 sessions
G
Reward Collection latency
Time (s)
Blocks of 2 sessions
H
Selection Trial Completion Rate
Selection Trials per Minute
Blocks of 2 sessions

Figure 4: Acquisition performance over time. 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 4,5, and 6, are composed of the remaining post-treatment sessions. All groups improved with successive sessions of PAL, completing more selection trials with higher accuracy, requiring fewer correction trials, as represented by significant main effects of Time. **[A]** Although there appeared to be a group difference in the number of selection trials performed, this trend was not significant. **[B]** For accuracy, there was an interaction, stress 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]** A significant interaction was found for total trials performed, both CORT and Vehicle animals performed more trials than control animals during block 1, which preceded any treatment. **[E-G]** In contrast to other measures, there was no improvement or decrement in latency over time. **[H]** All animals showed significant improvement over time on the selection trial completion rate. The stress group performed selection trials at a higher rate than controls on Blocks 3, 4 and 5, and at higher rate than the vehicle group on Block 5. * indicates pairwise comparison significant at $p < 0.05$.

4. DISCUSSION

Acute stress enhances acquisition of visuospatial behavioural tasks mediated through the DSTR, and we therefore hypothesized that both ARS and CORT would enhance performance in PAL. I found no effect of ARS or CORT on treatment day performance when compared to the control and vehicle groups. In contrast, during subsequent sessions, ARS animals improved more quickly in accuracy and selection trial completion rate when compared to controls. However, these effects were relatively small and not significantly different from the CORT and vehicle groups. Together these data suggest that acute stress does not impair same day performance, but that ARS facilitates acquisition of PAL.

4.1 No effect of acute stress on treatment day performance

The performance of animals was unaffected on all measures following CORT or ARS, when compared to the vehicle group and untreated controls. This suggests that in the short term, stress neither impairs nor enhances PAL performance during acquisition. We hypothesized that spatial memory impairments associated with acute stress would significantly affect performance 30 min after stress based on a large number of studies that have found similar results (de Quervain et al., 1998; Wright et al., 2006; Atsak et al., 2016). Therefore, it was surprising when there was no effect.

One possible explanation for the lack of treatment day effect may relate to competition between memory processes mediated through the DSTR and HPC. Previous studies have demonstrated that S-R behavioural strategies that rely on the DSTR are favoured following acute stress (Quirarte et al., 2009; Goodman et al., 2015). In PAL tested in mice, acquisition is sensitive to DSTR lesions and has been shown to proceed undeterred by HPC lesions (Delotterie et al., 2015). Acquisition was also unimpaired by HPC catecholamine depletion in rats (Roschlau

& Hauber, 2017). Conversely, PAL performance in well-trained animals is sensitive to HPC impairment (Talpos et al., 2009). Together, these data suggest the DSTR is essential during the initial stages of learning, while the HPC becomes involved in later task recall. In the present study, stress was delivered about midway through the acquisition period, when both memory systems may have been involved. This may have led to competing effects that are masking any overt effect of stress on recall by enhancing habitual behaviours. However, further experimentation will be required to determine whether this theory is correct.

4.2 Stress animals have increased accuracy and selection trial rate compared to controls in sessions following treatment

Although no difference was found during treatment day performance, animals subjected to ARS performed with significantly greater accuracy and completed selection trials at a greater rate when compared to controls in subsequent training sessions. We theorize that these effects are due to increased consolidation following stress. In contrast to the treatment day, subsequent sessions were not accompanied by stress and thus likely free of spatial recall impairments related to elevated hormone levels (Wright et al., 2006). This theory fits with previous studies in which acute stress promoted lasting structural and functional changes in rodents (Rocher et al., 2004; Uysal et al., 2012) and humans (Hermans et al., 2011). Furthermore, as ARS may enhance consolidation for both HPC-mediated and DSTR-mediated behaviours, a similar result could be produced regardless of the memory system involved. Thus, it appears that although no immediate effect was found following treatment, perhaps due to competing effects on consolidation and recall, acquisition was enhanced during subsequent trials.

However, one complication with the theory that acute stress improved acquisition of PAL through stress hormone-mediated consolidation is that no effect was seen following CORT treatment. This may be due to procedural differences between the two treatments, and the

relative engagement of parallel systems important to memory. ARS animals were immobilized for 30 min in a brightly lit novel room, which led to clear increases in fear behaviours such as piloerection and defecation. Following restraint, these animals were transported back to the touchscreen room where PAL began immediately. In contrast, for both the CORT and vehicle treatments, animals were brought to the novel room with lights dimmed, and given a single s.c. injection, which appeared to cause little discomfort, and was not accompanied by similar fear behaviours. The entire injection process took about 1 min, after which animals were returned to their home cages and left undisturbed for the remainder of the 30 min acclimatization period. As the effect of acute stress on consolidation relies on NA release and AMY activation (Roozendaal et al., 2004; Goode et al., 2016), one might predict that stresses that are more explicit or intense (e.g. restraint, footshock, predator odor) generate a greater response compared to injection alone.

Indeed, many studies have shown that while both ARS and 3.0 mg/kg CORT can produce similar spatial memory effects (Schwabe et al., 2010) this is not always the case (Mercier et al., 2003; Gregus et al., 2005). This appears to suggest a difference between the physiological response generated following an aversive event and the exogenous administration of stress hormones. Fear-induced CORT elevation impairs HPC-dependent spatial memory, leaving HPC-independent memory intact, while CORT elevation alone does not have an effect (Woodson et al., 2003). Therefore, we propose that ARS animals showed the greatest boost to acquisition as this procedure would produce an increase in corticosteroid levels as well as an increase in catecholamine release and emotional arousal. However, as we did not measure regional activities or hormone concentrations, this theory cannot be confirmed.

Interestingly, our study failed to identify any difference between animals treated with CORT or vehicle, which, although clearly not significant, appeared to perform better than controls on

several measures. One explanation may be that selection and experimental biases confounded these results. However, we believe there are several reasons to expect any effects associated with these biases to be minimal. First, all data collection and task operation in PAL is automated, preventing any potential effects arising from manual scoring. Secondly, animals were randomly assigned to groups, and experimenters were blind until treatment. And third, even following treatment, daily training was extremely prescriptive, with no opportunity for discretion and little direct involvement. Therefore, we expect that it is unlikely these effects were produced from bias alone.

However, there is a possibility that group differences were present between Squad 1 (ARS and control) and Squad 2 (CORT and vehicle). First, animals were not trained at the same time, with Squad 1 being trained months in advance of Squad 2. Second, although both squads were trained using the same protocol, CORT and vehicle animals were trained by two different researchers, while a single researcher trained all the ARS and control animals. It is possible that either of these changes produced slight differences in performance for both the CORT and vehicle groups, perhaps explaining significantly increased number of total trials in block 1 that caused them to outperform the controls. While we cannot discount this possibility, we found no difference in the number pre-treatment sessions required, and all groups had similar performance upon reaching criterion. Therefore, as an alternative, we suggest that both CORT and vehicle treated animals benefited, albeit more moderately than ARS, from CORT and injection stress. Indeed, we would not be the first group to show that injection of vehicle alone can produce behaviourally-relevant effects (Lipska et al., 1993; Belz et al., 2003) or promote S-R behaviours in spatial memory tasks (Schwabe et al., 2010; Atsak et al., 2016). Thus it is possible that both

injections slightly enhanced consolidation, but were insufficient to produce the same effect as ARS. Further experimentation would be required to confirm this.

4.3 Evaluation of PAL for further use in stress research

To the best of our knowledge this study is the first to explore the effects of acute stress on PAL. Although direct comparisons are difficult, these results fit well with previous work demonstrating that performance is unimpaired following treatment with low to moderate doses of common pharmacological manipulations such as phencyclidine (1.5mg/kg; Talpos et al., 2014), ketamine (5.0 mg/kg; Talpos et al., 2014), and amphetamine (0.25 mg/kg in Talpos et., 2014; 0.4 mg/kg in Roschlau et al., 2016). Furthermore, work in our lab has shown that PAL performance is preserved following moderate doses of the NMDA antagonist MK-801 (0.10 mg/kg; Lins et al., 2015). While many of these manipulations do not specifically target DSTR, they are commonly used as models known to effect memory and disrupt HPC function.

While this study did not provide clear evidence for a disassociation between the DSTR and HPC in PAL, the effect was not as large as we had expected. This is likely related to the many weeks required for pre-training and task acquisition. Further, although we were able to generate an effect with ARS, the limited response and failure of CORT suggests even moderate variations in stress are unlikely to seriously confound other studies. Taken together, these results suggest that PAL is not an ideal task to explore the effects of acute stress on striatal-dependent cognition, but does support continued use of PAL for further work in behavioural pharmacology. The minimal differences across groups suggests that in most instances a small amount of stress, such as that generated from an acute injection, is not likely sufficient to dramatically disrupt performance in this task.

4.4 Future Directions

Although the present experimental design did not produce as robust of a behavioural response as was desired, future research may refine this protocol to improve this in the future. The first step in improving this experimental design would be to evaluate how response to ARS differs based on the progression through acquisition training. The present study used a treatment timepoint, determined by pilot data, that placed animals approximately half way through acquisition. This timepoint was chosen to limit the potential of ceiling effects, and because previous research has shown that ARS has its greatest effect in the later stages of reversal learning (Bryce & Howland, 2015). Shifting treatment to an earlier session, and exploring the effects of multiple treatments, may yield a greater effect.

Additionally, future research should seek to determine if administration of ß-adrenergic antagonist (such as propranolol) is sufficient to negate the facilitation found in the ARS condition. Based on previous research which has found that many of the mnemonic effects of stress rely on NA release in the BLA (Roozendaal et al., 2004), one may expect that disrupting catecholamines during this task would prevent such a response. Alternatively, one may also explore the proposed requirement of catecholamine elevation in PAL through administration of yohimbine, or other such substrate, alongside CORT. Together these experiments would help determine whether the differences found during ARS, CORT, and vehicle treatments were indeed due to different levels of catecholamines

5. CONCLUSION

5.1 Acute restraint stress facilitates PAL acquisition, but CORT and injection stress do not

Based on previous research that has found that acute stress enhances S-R learning, we predicted that a single session of acute restraint stress or a single injection of CORT would facilitate the acquisition of touchscreen-based visuospatial PAL in rats. However, although animals subjected to ARS performed more accurately and had a greater selection trial completion rate compared to control animals, performance did not differ between the ARS, CORT, and vehicle-treated animals. The effect of restraint stress, although significant statistically, was relatively small. Additionally, we found no effect of CORT on any measure explored compared to vehicle or control animals. This may have been due to the dose of CORT used (3.0 mg/kg), or may be indicative of differences between these stresses and the level of emotional arousal produced, which has proven important in other studies.

This research may provide some support for previous work that has identified a prominent role of the DSTR in the acquisition of PAL. However, whether the enhanced acquisition of PAL observed following restraint stress is due to an increase in DSTR-mediated S-R learning or enhanced HPC consolidation cannot be confirmed by this study. In closing, although the limited effects found in this study do not support using PAL for future acute stress research, it does suggest that this task may be resistant to variations caused by stress, which may be valuable for future studies in which effects of acute stress may otherwise confound results.

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