# Title: Procedural learning under stress: boosted statistical learning but unaffected sequence learning

## Running title: Procedural learning under stress

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**Abstract**

Acute stress can crucially influence learning and memory processes. A growing body of evidence shows that stress can induce a shift from cognitively demanding goal-directed to habitual, procedural forms of learning. However, the mechanisms underlying the shift are not yet understood. Here, we aimed to get an in-depth look into the effect of stress on the acquisition of procedural information with disentangling two aspects of procedural learning. We examined whether and how stress affects (1) processes and learning of probability-based information and (2) serial order-based information in the same experimental design, and (3) explicit access to the acquired information. Considering statistical learning, based on prior results, we primarily focused on the early phases of the task. We induced stress with the Socially Evaluated Cold Pressor Test in 27 young adults, while 26 participants were enrolled in the control group. Salivary cortisol levels and subjective ratings of affective states showed successful stress induction. After the stress induction, we measured procedural learning with the cued Alternating Serial Reaction Time task. We found that stress promoted the acquisition of probability-based information and did not alter the learning of order-based information. Post-block reports showed weaker explicit access to the order-based information in the stress group. Our results are in line with the theory of stress-induced memory shift and give a process-level understanding on how stress alters procedural learning.

Keywords: procedural learning, stress, cortisol, statistical learning, deterministic rule-based learning

## Introduction

Stressful situations are ubiquitous parts of our everyday life. Acute stress leads to changes in both cognitive and affective processes (Joëls et al., 2011; Schwabe et al., 2012; Ehlers and Todd, 2017; Dolfen et al., 2019). Previous studies have shown the crucial influence of stress on learning and memory systems. Stress and glucocorticoid hormones (cortisol in humans) that are released in stressful situations can induce a shift from cognitively demanding, goal-directed to habitual forms of learning (e.g. Schwabe et al., 2007; Schwabe and Wolf, 2010, 2011, 2013; Fournier et al., 2017; Wirz et al., 2018). Although there is a growing body of evidence for this shift, the exact underlying mechanisms are still poorly understood. The aim of the present study was to investigate the effect of stress on habitual forms of learning to gain better understanding of stress-induced shift between memory systems.

Although stress-induced memory shift has been widely demonstrated, it is still unclear whether this shift results from disrupted goal-directed or enhanced habitual processes, or a combination of both. For example, Fournier et al. (2017) reported disrupted goal-directed and unaffected habitual performance on a behavioral instrumental task following stress induction, whereas Schwabe et al. (2012) associated stress-induced memory shift with reduced activity in the orbitofrontal and prefrontal cortex, regions involved in goal-directed behavior. Importantly, however, stress may also alter habit learning (Wood and Rünger, 2016). Studies investigating the effect of stress on the habitual processes in humans yielded inconsistent results (Kirschbaum et al., 1996; Schwabe and Wolf, 2012; Guenzel et al., 2014a; 2014b).

In the present study, we conceptualized habit learning as a form of procedural learning (Squire et al., 1993). Habit learning and procedural learning are not unified processes; instead, they contain several types of learning. It is possible that stress influences these aspects of learning differently. Note that habit learning as a form of procedural learning vs. as a form of

instrumental learning (as conceptualized in previous studies examining stress-induced memory shift) are highly compatible (Seger, 2018). According to both conceptualizations, one acquires some knowledge through repeated presentation, and with practice, the acquired knowledge becomes automatic and less cognitively demanding (Ashby et al., 2010; Ashby and Crossley, 2012; Ullman et al., 2020).

To test the effect of stress on habit learning, we used the cued version of the Alternating Serial Reaction Time (ASRT, Howard and Howard, 1997; Németh et al., 2013) task that enables us to measure two aspects of learning, namely statistical learning and sequence learning simultaneously. Here, we use statistical learning in a narrow sense, it refers to the acquisition of shorter-range relation between visual stimuli that is mainly based on probability-based information (i.e. differentiating between more and less probable stimuli). Sequence learning refers to the acquisition of serial order-based information, that is, participants learn a series of stimuli that repeatedly occur in the same (deterministic) order, alternating with random stimuli. We can view this type of learning as a deterministic rule learning (Maheu et al., 2020). Statistical and sequence learning are distinguishable on both behavioral and neural levels: they show different neural oscillations during consolidation (Simor et al., 2019), and dissociation in neurodevelopmental disorders (i.e. Tourette syndrome; Tóth-Fáber et al., under review). On the behavioral level, participants acquire probability-based information rapidly and show consistent, stable performance thereafter, while sequence learning progresses more gradually (Kóbor et al., 2018; Simor et al., 2019).

The aim of the present study thus was to test the effect of stress on two aspects of procedural learning, namely statistical and sequence learning. As prior studies are scarce and inconclusive, we use an exploratory approach to investigate this question. As statistical learning is a rapid process, if stress alters this aspect of learning, we would expect it to do so in the early phase of the task. Therefore, we examined whether stress affects the extraction of

the presented statistics at the beginning of the task. Considering sequence learning, as it shows a gradual trajectory, if stress affects it, we would expect alterations in the later stages of the task.

## Materials and methods

### Participants

The final sample consisted of 53 participants (15 men, 38 women; $M_{age}$ = 20.9 years, SD = 1.7). Participants were randomly assigned to either the control (7 men, 19 women) or the stress (8 men, 19 women) group. All participants had normal or corrected-to-normal vision, none of them reported a history of any psychiatric, neurological or any other chronic medical problems. They performed in the normal range of standard neuropsychological tests (Counting Span task: $M_{stress}$ = 3.87, $M_{control}$ = 3.73; Digit Span task: $M_{stress}$ = 7.03, $M_{control}$ = 7.23), with no difference between the stress and control groups (all *p*s > .537, Cohen's *d* < 0.176).

Participants were recruited from a university course in exchange for course credit. All participants provided written informed consent before enrolment. The study was approved by the research ethics committee of the Eötvös Loránd University in Hungary and was conducted in accordance with the Declaration of Helsinki. For additional information about participants, see Supplementary Material.

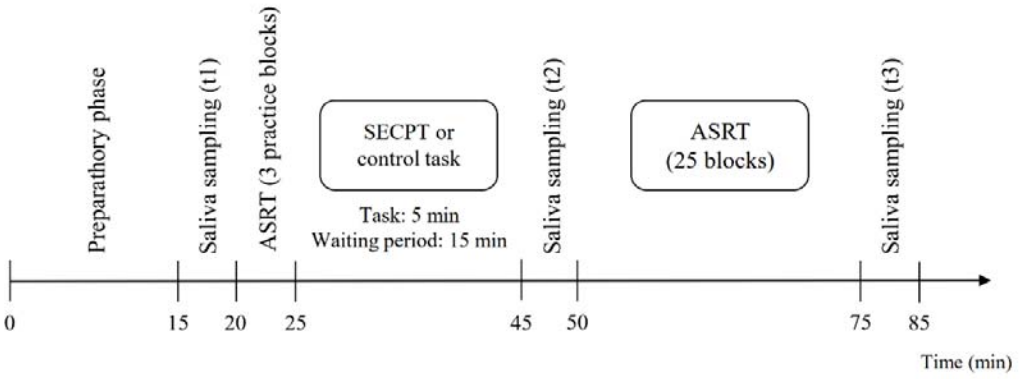


**Figure 1. Experimental procedure.** Cortisol levels were assessed from saliva at three time points: immediately before the stress induction or control task (t1), 15 minutes after the stress induction or control task (t2), and immediately after the ASRT task, ca. 45 minutes after the stress induction or control task (t3). ASRT: Alternating Serial Reaction Time task; SECPT: Socially Evaluated Cold Pressor Test.

## Experimental design

To exclude the effect of critical factors on salivary cortisol levels, participants were asked not to eat, drink (except for water), smoke, and make physical exercise two hours prior to the experiment (see Kirschbaum and Hellhammer, 1994; Lovallo et al., 2006; Badrick, Bobak, et al., 2007; Badrick et al., 2007). To avoid interference with the cortisol circadian cycle (see Clow et al., 2010), assessments took place between 1 and 7 p.m.

Each experimental session started with a 15-min preparatory phase while participants remained in a preparatory room for 15 minutes to minimize the effect of potentially stress-inducing factors (such as new environment; see Lupien et al., 2007). To measure cortisol levels, saliva samples were collected at different time points during the experimental session (see Figure 1). The first saliva sampling was conducted immediately after the preparatory phase. Then, participants completed the practice session of the procedural learning task, followed by the stress induction or control task (see the details below). To assess subjective stress levels, immediately after the stress induction or the control task, participants were

required to rate how stressful (0 = not at all, 100 = very stressful), painful (0 = not at all, 100 = very painful), and unpleasant (0 = not at all, 100 = very unpleasant) the stress inducing or the control task was (Table 1). The second saliva sample was collected 15 minutes after stressor offset/the end of the control task, followed by the procedural learning task. The third saliva sample was collected immediately after the procedural learning task (approximately 40 minutes after stressor offset/the end of the control task). Samples were collected using Eppendorf Safe-Lock Tubes (1.5 mL) and were analyzed using Salimetrics immunoassays.

**Stress induction task and control condition**

Individuals in the stress group participated in the Socially Evaluated Cold Pressor Test (Schwabe et al., 2008). This task was developed to induce moderate stress in laboratory settings using both physical and psychosocial stressors. Participants were instructed to put their hand into cold water (0-3 °C) for three minutes while a female experimenter observed their behavior. The observer did not interact with the participants, just passively monitored their behavior. Participants were also told that video recording would be made to later analyze their non-verbal behavior. They were informed that they could interrupt the task if it would be too painful or uncomfortable.

There were no stress inducing factors (i.e. cold water, observer, and video recorder) in the control condition. Control participants were required to put their hand into warm water (35-37 °C) for three minutes. A female experimenter stayed in the experimental room (just as in the stress condition), however, this time she did not observe participants' behavior.

**Procedural learning task**

Procedural learning was measured by the cued version of the Alternating Serial Reaction Time (ASRT) task (Song et al., 2007; Németh et al., 2013; Simor et al., 2019). In

this task, a target stimulus (either a dog's head or a penguin) appeared in one of the four possible locations, which were horizontally arranged and equally spaced empty circles. Participants were asked to press the corresponding key on the keyboard (Z, C, B, or M) as accurately and as fast as they could. The stimulus remained on the screen until the participant's response, then, after a 120 ms delay, the next target appeared.

The presentation of the stimuli followed an 8-element alternating sequence where pattern and random elements alternated with each other (e.g. 1-r-2-r-4-r-3-r, where numbers represent the predetermined locations from left to right and 'r' indicates a randomly selected location). The pattern and random elements were marked differently, pattern elements indicated by the dog's head, and random elements represented by the penguins. Participants were told that the appearance of the dogs always followed a predetermined sequence, while penguins always appeared on a randomly chosen location. They were instructed to find the pattern defined by the dog's appearance in order to improve their performance. They had no information about the length of the predetermined sequence. The alternation of pattern and random elements creates six distinguishable sequence permutations: 1-r-2-r-3-r-4-r, 1-r-2-r-4-r-3-r, 1-r-3-r-2-r-4-r, 1-r-3-r-4-r-2-r, 1-r-4-r-2-r-3-r, and 1-r-4-r-3-r-2-r. One of these permutations was selected for each participant and it was counterbalanced across participants in each group.

The task contained 20 blocks, each block consisted of 85 trials. The first five trials of each block were random trials for practice, then the unique 8-element predetermined sequence was presented 10 times. After each block, participants were instructed to type the order of the dog's head using the corresponding keys to measure awareness of the sequence structure. The *post-block sequence report* lasted until the participants gave 12 consecutive responses, which are ideally the 4-element sequence three times. For these post-block sequence reports, we calculated how many responses out of the total 12 were correct after each block, expressed in

terms of percentages. The mean of these block-level values was calculated for each participant and used as a measure of their *explicit sequence knowledge* acquired during the task.

The alternating sequence in the ASRT task makes some runs of three successive elements – referred to as *triplets* – more probable than others. For example, if the sequence is 1-r-2-r-4-r-3-r, triplets such as 1-X-2, 2-X-4, 4-X-3, 3-X-1 (where X indicates the middle element of the triplet) occur frequently since their last element can be either predetermined or random. However, 3-X-2 or 4-X-2 occur less often as the third element could only be random. The more probable triplet types are hereinafter referred to as high-probability triplets and the latter types as low-probability triplets (Howard and Howard, 1997; Németh et al., 2013). Since high-probability triplets can end with a pattern trial due to the predetermined sequence or with a random trial by chance, we can differentiate between pattern high-probability and random high-probability triplets. Low-probability triplets can only end with a random trial, as pattern trials are always at high probability. Note that each element (i.e., trial) was categorized as the third element of either a high-probability or a low-probability triplet. In sum, three trial types can be differentiated: (1) trials that belong to the predetermined sequence and are the final element of a high-probability triplet labeled as *pattern* trials, (2) trials that occur randomly and also are the final element of a high-probability triplet called *random high* trials, and (3) random elements that appear as the last element of a low-probability triplet called *random low* trials.

Based on these trial types, we can differentiate between two types of procedural memory: statistical learning and sequence learning (Figure 2; Howard & Howard, 1997; Németh et al., 2013; Kóbor et al., 2018; Simor et al., 2019). *Statistical learning* refers to the acquisition of probability-based information, it is measured by the difference in reaction times (RTs) between random high and random low trials. These trials share the same sequence

structure as they are both random trials, but differ in statistical properties as they correspond either to the final trial of a high-probability or a low-probability triplet. Greater statistical learning is indicated by faster RTs on random high than on random low trials. *Sequence learning* refers to the acquisition of serial order-based information, it is measured by the difference in RTs between pattern and random high trials. These trials share the same statistical properties as they are both the last element of a high-probability triplet, but differ in sequence properties as pattern trials are part of the predetermined sequence, while random high trials appear on a random location. Greater sequence learning is defined as faster RTs on pattern than on random high trials.

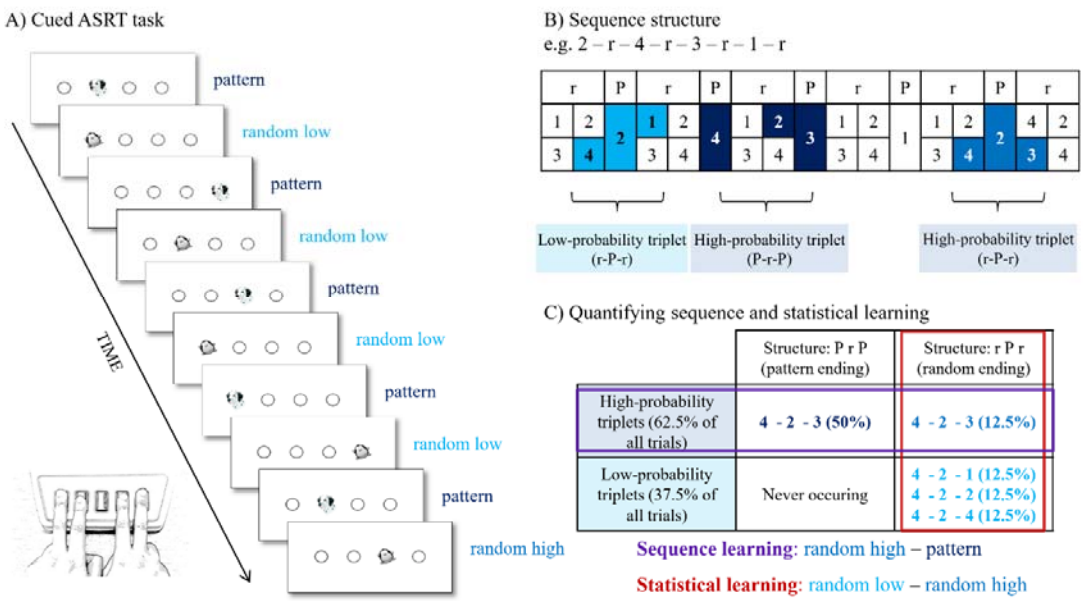


**Figure 2. The cued Alternating Serial Reaction Time (ASRT) task.** (A) Pattern and random trials were presented in an alternating fashion in the ASRT task. Pattern trials were indicated by a picture of a dog and random trials were indicated by a picture of a penguin. (B) An example of the alternating sequence structure. Here, numbers indicate pattern elements and 'r' indicates a randomly selected location. The alternating sequence makes some runs of three consecutive trials (called triplets) more probable than others, termed high- and low-probability triplets, respectively. Among high-probability triplets, we determined pattern high-probability triplets (with dark blue shading in panel B and dark blue font in panel C) and random high-probability triplets (with blue

shading in panel B and blue font in panel C). Only random low-probability triplets can occur (with light blue shading in panel B and light blue font in panel C). (C) The underlying learning processes in the task. Sequence learning is quantified by contrasting the RTs on the pattern and random high trials (dark blue vs. blue, top row of the table). Statistical learning is quantified by contrasting the RTs on the random high and random low trials (blue vs. light blue, right column of the table).

## Statistical analysis

Statistical analyses were carried out by SPSS version 25.0 software (SPSS, IBM). As participants were instructed to find the alternating sequence and report that during the task, performance on the sequence report (calculated as the average percentage of correctly reporting the sequence after the ASRT task blocks) could show whether participants adequately followed the instructions of the task. In the present study, seven participants exhibited below 50% performance, suggesting that they could not follow the instructions properly and were therefore excluded from the analyses (see also the Participants section; for analysis on the sample containing these participants, see Supplementary Material).

Statistical analysis of the effectiveness of stress induction was the same as in prior studies (e.g. Guenzel et al., 2014a, Szőllősi et al., 2017). We conducted a mixed design ANOVA on cortisol levels as dependent variable, with TIME (t1, t2, and t3) as a within-subject factor, where t1 = immediately before stress/control procedure, t2 = 15 minutes after stress/control procedure, t3 = 45 minutes after stress/control procedure, and with STRESS EXPOSURE (stress or control) as a between-subject factor. The effectiveness of stress induction was also quantified with subjective variables. Immediately after the stress induction or control task, participants were asked to rate how stressful, painful, and unpleasant the stress induction or the control task was. We compared these subjective ratings of affective state between the stress and control groups with independent sample t-tests.

Statistical analysis of the ASRT task was based on previous studies (Németh et al., 2013; Kóbor et al., 2018; Simor et al., 2019). Two types of low-probability triplets, namely repetitions (e.g. 111, 222) and trills (e.g. 121, 242) were excluded from the analysis, since participants often show pre-existing tendencies to these trials (Song et al., 2007a, 2007b). Following prior studies (e.g. Németh et al., 2013; Janacsek et al., 2018; Kóbor et al., 2017; 2018; Simor et al., 2019; Takács et al., 2018), the 1-minute-long blocks were organized into larger segments labeled as epochs. Each epoch consists of 5 blocks, thus, we analyzed five epochs of the ASRT task.

As described in the Procedural learning task section, each trial was determined as the last element of a pattern high, random high, or random low triplet. The median of RT data for correct responses was calculated for each participant in each epoch, separately for the three trial types. On average, in the statistical learning analysis, the stress group showed faster RTs compared to the control group. To ensure that potential group differences in statistical learning or sequence learning are not a result of general RT differences, we transformed the data in the following way. We divided each participants' raw RT values of each trial type and each epoch by their own average performance of the first epoch of the task (for a similar approach, see Nitsche et al., 2003; Horváth et al., 2019), then we multiplied these values by 100 for easier presentation and interpretability. For analysis on raw RT data, see Supplementary Material.

As presented above, two aspects of procedural learning, sequence learning and statistical learning can be assessed by the ASRT task (Németh et al., 2013; for further details, see Introduction and Methods). Sequence learning was calculated by extracting standardized RTs to pattern trials from RTs to random high trials. Statistical learning was calculated by extracting standardized RTs to random high triplets from RTs to random low triplets. Due to the transformation procedure, the learning scores can be interpreted as percentages, showing

how much faster participants responded to pattern vs. random high trials (sequence learning) or to random high vs. random low trials (statistical learning). In both cases, higher learning scores indicate better learning. At first, as statistical learning is a rapid process (Kóbor et al., 2018; Simor et al., 2019), we focused on the early phase of the task. We used independent sample t-test to compare statistical learning scores between the stress and control groups in the first epoch of the task. Similarly, at first, sequence learning was compared only in the last epoch of the task between the groups as this learning process shows a gradual trajectory (Kóbor et al., 2018; Simor et al., 2019). To investigate learning on the whole task, RT data were analyzed in mixed design ANOVAs separately for sequence learning and statistical learning scores, with EPOCH (1-5) as a within-subject factor and GROUP (stress vs. control) as a between-subject factor. Pairwise comparisons were performed using LSD (Least Significant Difference) to control for type 1 error. The Greenhouse-Geisser epsilon correction was used when necessary. Corrected *df* values and corrected *p* values (if applicable) are reported together with partial eta-squared ($\eta^2_p$) as a measure of effect size.

## Results

### *The effectiveness of stress induction*

We used both objective (cortisol levels) and subjective (questionnaire-based rating) measurements to test the effectiveness of stress induction. For the *objective measurement*, the ANOVA confirmed the effectiveness of the stress induction. We found a significant TIME × STRESS EXPOSURE interaction ($F(2, 102) = 29.75$, $p < .001$, $\eta^2_p = .368$). The post hoc analysis revealed that cortisol levels in the stress and control groups differed 15 minutes after the stress/control procedure (t2; $M_{\text{stress}} = 32.62$ and $M_{\text{control}} = 22.26$, respectively, $p = .048$), whereas immediately before the stress/control procedure (t1) and immediately after the ASRT task (t3) the two groups' cortisol levels did not differ significantly (both *p*s > .34, see Figure

3). For the *subjective ratings* of affective state after stress induction or control task, the t-tests showed significantly higher subjective stress, pain, and unpleasantness levels in the stress group compared to the control group (see Table 1).

**Table 1. Descriptive data of the participants' subjective stress measurements.**

| | Group | | | | | |
|---|---|---|---|---|---|---|
| | Stress ($n$ = 26) | | Control ($n$ = 26) | | | |
| | *M* | *SD* | *M* | *SD* | *t* | *p* |
| Subjective ratings of stress | 29.88 | 26.14 | 8.34 | 12.43 | -3.85 | <.001 |
| Subjective ratings of unpleasantness | 56.29 | 28.78 | 11.30 | 14.29 | -7.24 | <.001 |
| Subjective ratings of painfulness | 58.14 | 25.56 | 1.34 | 5.92 | -11.23 | <.001 |

*Note*. Immediately after the stress induction or control task, participants rated how stressful, unpleasant, and painful (where 0 = not at all, 100 = very) the stress induction or control task was.

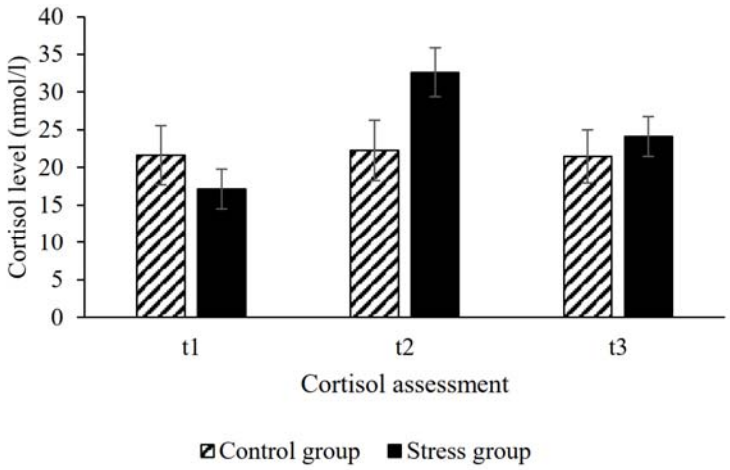

**Figure 3. The effectiveness of stress induction.** Salivary cortisol levels were assessed immediately before (t1), 15 minutes after (t2), and 45 minutes after (t3) the stress induction or control task. Error bars denote standard error of mean.

### *The effect of stress induction on statistical learning*

As statistical learning is a rapid process (Kóbor et al., 2018; Simor et al., 2019), we focused on the beginning of the task. At first, we compared the statistical learning scores (see Data analysis) between the stress and control groups in the first epoch of the task using independent samples t-test. The analysis revealed a significant difference between the groups ($t(51) = -2.11$, $p = .040$), with higher learning in the stress group ($M_{\text{stress}} = 4.85$, $M_{\text{control}} = 2.25$).

To investigate statistical learning in the stress and control groups on the whole task, we conducted a mixed-design ANOVA on the statistical learning scores (see Data analysis). Overall, the ANOVA revealed significant statistical learning (shown by the significant INTERCEPT ($F(1, 51) = 142.04$, $p < .001$, $\eta^2_p = .736$). Statistical learning occurred relatively rapidly (as typically observed, Kóbor et al., 2018; Simor et al., 2019) and did not change significantly as the task progressed (main effect of EPOCH, ($F(4, 204) = 1.70$, $p = .151$). Overall, the stress group showed marginally greater statistical learning scores ($M = 5.27$) than the control group ($M = 3.85$), as indicated by the marginally significant main effect of GROUP ($F(1, 51) = 3.45$, $p = .069$; $\eta^2_p = .063$, Figure 4). The EPOCH × GROUP interaction did not reach significance ($F(4, 204) = 1.38$, $p = .24$).

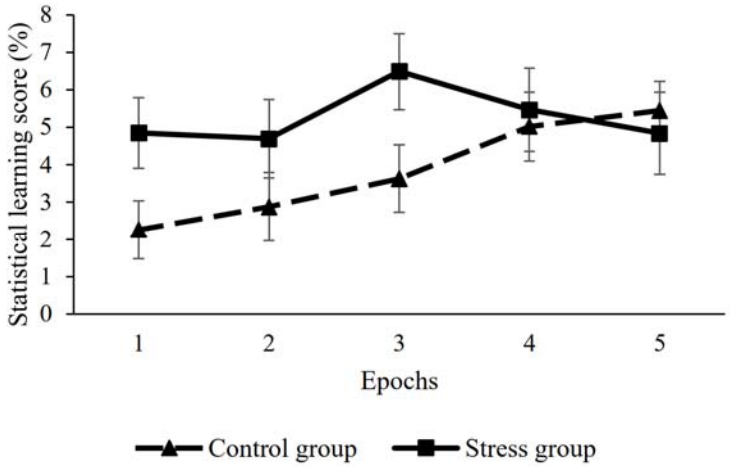


**Figure 4. Statistical learning in the stress and control groups.** Statistical learning score was computed by extracting RTs of the random high triplets from RTs of the random low triplets, separately in each epoch. Higher learning score indicates better learning. A significant difference was found between the two groups in the first and third epochs, with the stress group showing greater statistical learning scores in both epochs (1st epoch: $M_{stress}$ = 4.85, $M_{control}$ = 2.25, $p$ = .040; 3rd epoch: $M_{stress}$ = 6.49, $M_{control}$ = 3.63, $p$ = .041; in the remaining epochs $p$s > .198). Error bars denote standard error of mean.

### *The effect of stress induction on sequence learning*

As sequence learning is a gradual process (Kóbor et al., 2018; Simor et al., 2019), at first, we compared the sequence learning scores (see Data analysis) between the stress and control groups in the last epoch of the task using independent sample t-test. The analysis showed comparable sequence learning between the groups ($t$(51) = 0.77, $p$ = .44, $M_{stress}$ = 9.58, $M_{control}$ = 15.96).

To compare sequence learning between the two groups on the whole task, we conducted a similar mixed design ANOVA on the sequence learning scores (see Data analysis). Overall, the ANOVA revealed significant sequence learning (shown by the significant INTERCEPT ($F$(1, 51) = 14.37, $p$ < .001, $\eta^2_p$ = .220). Sequence learning increased as the task progressed (indicated by the significant main effect of EPOCH, $F$(1.3, 67.9) =

4.75, $p$ = .023, $\eta^2_p$ = .085). The ANOVA revealed no significant group differences in sequence learning (indicated by the non-significant main effect of GROUP, $F(1, 51) = 0.35$, $p$ = .55, and EPOCH × GROUP interaction, $F(1.3, 67.9) = 0.42$, $p$ = .57, Figure 5).

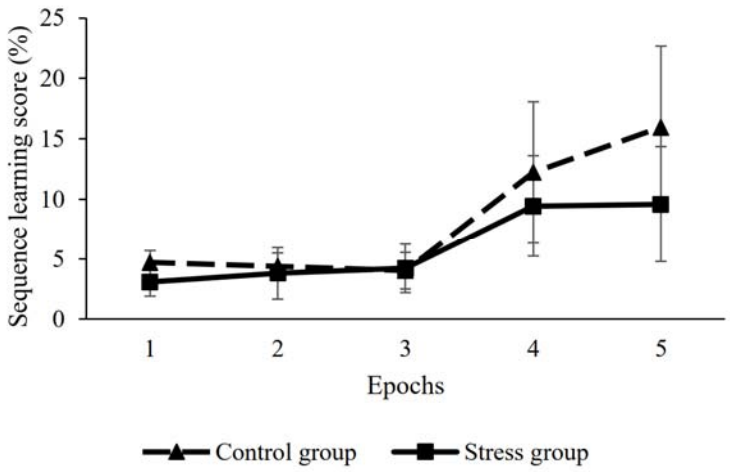


**Figure 5. Sequence learning in the stress and control groups.** Sequence learning score was computed by extracting RTs of the pattern high trials from RTs of the random high trials, separately in each epoch. Higher learning score indicates better learning. No significant difference was found between the groups in either epoch (all *p*s > .299) Error bars denote standard error of mean.

Performance on *post-block sequence reports*, reflecting explicit sequence knowledge, was also compared between the groups. The independent samples t-test showed that explicit sequence knowledge was higher in the control group than in the stress group ($t(42.5) = 2.12$, $p$ = .039, $M_{\text{control}}$ = 96.2%, $M_{\text{stress}}$ = 92.4%,). As a next step, we tested whether explicit knowledge about the sequence on the post-block sequence report was related to sequence learning performance measured by RTs, separately in the control and in the stress group. Four participants in the control group and four participants in the stress group showed extremely high sequence learning score according to Tukey's (1977) criterion (more than 1.5 times the interquartile range). We excluded these participants from the correlation analysis. Correlation

between explicit sequence knowledge and sequence learning scores did not reach significance either in the control ($r_s(20) = .07$, $p = .74$) or in the stress group ($r_s(21) = .29$, $p = .17$).

**Discussion**

To date, several studies have found a stress-induced shift from goal-directed to habitual, procedural learning processes (e.g., Schwabe et al., 2007; Schwabe and Wolf, 2010, 2011, 2013; Fournier et al., 2017; Wirz et al., 2018), the underlying mechanisms, however, are still unclear. In the present study, we aimed to get a better understanding of procedural learning under stress. We went beyond previous studies by examining procedural learning not as a homogenous learning process but measuring two aspects of it: probability-based statistical learning and serial order-based sequence learning. Our results showed that stress affected the acquisition of probabilistic information, while sequence learning was unaffected by the stress induction. Moreover, the stress group showed lower explicit sequence knowledge of the sequence structure presented in the task. Thus, stress promoted an aspect of procedural learning to some extent while disrupted explicit processes.

Considering statistical learning, prior studies examining the acquisition of some kind of probabilistic structure under stress are scarce. Schwabe and Wolf (2012) investigated probabilistic classification after experimental stress induction. Stress did not alter probabilistic classification performance, however, based on the debriefing after the task, participants in the stress group favored procedural strategies at the expense of declarative, explicit strategies. In the present study, stress altered behavioral performance, somewhat boosting the acquisition of probabilistic information. Both studies suggest that stress boosts procedural learning, which fits well into the theory of stress-induced memory shift (Schwabe & Wolf, 2013). Importantly, our study further strengthens this notion with demonstrating the effect of stress

on behavioral performance, well complementing prior studies' results from subjective post-task strategies (Schwabe and Wolf, 2012).

It seems that stress induction had a more robust effect on the acquisition of probabilistic information at the beginning of the task. There are two possible explanations for this effect, which, at this point, cannot be discriminated. First, acquiring statistical information occurs rapidly, often in the early phase of the task, therefore, it is possible that stress boosted the *acquisition* of statistical information but not the stabilization of the acquired knowledge. However, as indicated by the cortisol levels, stress had a shorter effect than the duration of the ASRT task. Our analysis showed that cortisol levels were comparable immediately after the ASRT task between the groups, meaning that during the task, cortisol levels in the stress group decreased to the level of the control group. As we did not track cortisol levels during the task, we cannot determine in what pace the cortisol levels changed in the stress group. We can only speculate that it decreased gradually as the task progressed. With this information on hand, we cannot take stand on either explanation, i.e. whether stress only boosts the acquisition but not the stabilization of statistical information or stress induction only had a short-term effect, which resulted in group differences only in the early phase of learning. Further studies are needed to directly test this possibility with assessing cortisol levels during the task as well.

Studies have examined the effect of stress on habitual performance using tasks tapping into procedural functions, such as sequence learning (Roemer et al., 2011; Schwabe et al., 2009) or probabilistic classification (Schwabe & Wolf, 2012). Considering sequence learning, prior studies are not conclusive. Roemer et al. (2011) used oral cortisol to induce stress in a subset of their participants, then measured sequence learning with the Serial Reaction Time (SRT) task. Participants learned higher-order sequential regularities (i.e. how the combination of two colors predicted the following third target color). Altogether, the cortisol group showed

comparable learning compared to the control group. At the beginning of the task, the cortisol group showed lower learning scores than the control group, then they showed similar performance, suggesting that higher cortisol levels delayed the acquisition of sequential regularities. Our results partly fall in line with these findings, as we have found intact, comparable sequence learning in the stress group compared to the control group. However, it is important to note that previous studies are sparse and the results are inconsistent, as some studies showed improved sequence learning due to higher cortisol levels (Roemer et al., 2009), while Schwabe et al. (2009) showed no effect of stress on the retention of sequential regularities. Moreover, it should be taken into consideration that the Roemer et al.'s (2011) study used psychopharmacological stress induction, therefore, the results may not be clearly transferred into the stress context. In sum, further studies are warranted to investigate the effect of stress on the acquisition of sequential regularities in the context of both psychopharmacological and experimental stress induction.

Note that the cued version of the ASRT task used in this study (Kóbor et al., 2018; Simor et al., 2019) enables us to assess statistical and sequence learning within one learning session. The acquisition of probabilistic information occurs incidentally (i.e. without awareness or intention to learn) and relatively rapidly (Kóbor et al., 2018; Simor et al., 2019). In contrast, the acquisition of the alternating sequence shows a gradual trajectory and it can be supported by incidental and intentional learning processes as well, with a typically faster acquisition in the intentional learning condition compared to an incidental learning condition (Howard & Howard, 1997; Howard et al., 2004; Simor et al., 2019). In the cued version of the task, pattern and random elements are visually distinguishable, and participants are instructed to find the sequence, which creates a somewhat intentional learning condition. However, it is important to note that even in an intentional learning situation, RTs tend to reflect a more implicit, incidental measure of sequence knowledge, while explicit sequence knowledge tends

to reflect a more intentional, goal-directed process. As typical response times are under 500 ms, the fast pace of the task makes it unlikely that explicit sequence knowledge would have an impact on participants' RTs. Therefore, the consciously accessible knowledge of the sequence (reflected by the sequence report score) and sequence learning (reflected by the RTs) may be somewhat dissociable measures (Horváth et al., 2019). The sequence report may be considered as a more explicit measure of sequence knowledge, while the sequence learning scores may serve as an implicit measure of sequence acquisition, even in an intentional learning condition. In this view, in the present study, stress disrupted the explicit acquisition of the sequence knowledge, while did not alter the implicit measure of sequence learning.

Considering the stress-induced memory shift, it is important to note that concurrent glucocorticoid and noradrenergic activity are needed for the memory shift (Schwabe et al., 2010; 2011; 2012). For this reason, we used the Socially Evaluated Cold Pressor Test (SECPT) containing both physical and psychosocial stressors, which are necessary for the simultaneous noradrenergic and glucocorticoid activity. Our results showed a significant glucocorticoid activity (i.e. increased cortisol levels in the stress group), however, one limitation in our study is that we could not measure alpha-amylase activity (that is a reliable marker of noradrenergic activity; see Nater et al., 2005). Nevertheless, SECPT is a reliable tool to induce both glucocorticoid and noradrenergic activity (Schwabe et al., 2008) and has been shown to induce the shift from the goal-directed to the habitual memory system (Schwabe & Wolf, 2010). Therefore, we can strongly assume that besides the increased glucocorticoid activity assessed with salivary cortisol levels, we also induced concurrent noradrenergic activity using the SECPT.

There are some methodological differences between the present and previous studies that should be taken into account when interpreting results. As mentioned briefly above, there are differences between administrating stress experimentally and pharmacologically.

Pharmacological manipulation often leads to higher glucocorticoid concentration than a stress induction task (Roozendaal et al., 2006; Schwabe et al., 2012). Moreover, stress induction tasks can increase not only the level of cortisol but also trigger the release of many other hormones and neurotransmitters, which can also influence learning (Contarino et al., 1999). Furthermore, pharmacological manipulation does not result in concurrent noradrenergic activity besides glucocorticoid activity, which occurs, however, after inducing stress experimentally (Schwabe et al., 2008). In sum, further studies are warranted to systematically investigate and contrast the effect of stress on different procedural (habit) learning tasks, while taking into account the type of stress administration.

In sum, the present study investigated whether and how two aspects of procedural learning, namely statistical learning and sequence learning, are influenced by stress induction. Our results showed that stress affected the acquisition of probabilistic information as participants in the stress group showed higher statistical learning compared to the control group. Stress did not alter serial order-based sequence learning but disrupted the explicit acquisition of the repeating sequence. Our results fit well into the notion of stress-induced memory shift, but also goes beyond prior studies by examining two aspects of procedural learning. We can also interpret our results from an evolutionary point of view: in a risky, dangerous situation, the human brain extracts probabilistic information embedded in our environment faster. This stress-related boost provides an advantage for the individual to effectively manage the situation.

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## References


Ashby FG, Crossley MJ (2012) Automaticity and multiple memory systems. Wiley Interdisciplinary Reviews: Cognitive Science 3(3):363-376.

Ashby FG, Turner BO, Horvitz JC (2010) Cortical and basal ganglia contributions to habit learning and automaticity. Trends in cognitive sciences 14(5):208-215.

Badrick E, Bobak M, Britton A, Kirschbaum C, Marmot M, Kumari M (2007) The relationship between alcohol consumption and cortisol secretion in an aging cohort. The Journal of Clinical Endocrinology and Metabolism, 93:750–757.

Badrick E, Kirschbaum C, Kumari M (2007) The relationship between smoking status and cortisol secretion. The Journal of Clinical Endocrinology and Metabolism, 92:819–824.

Clow A, Hucklebridge F, Stalder T, Evans P, Thorn L (2010) The cortisol awakening response: More than a measure of HPA axis function. Neuroscience and Biobehavioral Reviews, 35:97–103.

Contarino A, Dellu F, Koob GF, Smith GW, Lee KF, Vale W, Gold LH (1999) Reduced anxiety-like and cognitive performance in mice lacking the corticotropin-releasing factor receptor 1. Brain research 835(1):1-9.

Dolfen N, King BR, Schwabe L, Swinnen S, Albouy G (2019) Glucocorticoid response to stress induction prior to learning is negatively related to subsequent motor memory consolidation. Neurobiology of learning and memory 158:32-41.

Ehlers MR, Todd RM (2017) Acute psychophysiological stress impairs human associative learning. Neurobiology of learning and memory 145:84-93.

Fournier M, d'Arripe-Longueville F, Radel R (2017) Effects of psychosocial stress on the goal-directed and habit memory systems during learning and later execution. Psychoneuroendocrinology 77:275-283.

Guenzel FM, Wolf OT, Schwabe L (2014a) Sex differences in stress effects on response and spatial memory formation. Neurobiology of Learning and Memory 109:46-55.

Guenzel FM, Wolf OT, Schwabe L (2014b) Glucocorticoids boost stimulus-response memory formation in humans. Psychoneuroendocrinology 45:21-30.

Horváth K, Torok C, Pesthy O, Nemeth D, Janacsek K (2019) Intention to learn differentially affects subprocesses of procedural learning and consolidation: Evidence from a probabilistic sequence learning task. bioRxiv 433243.

Howard JH, Howard DV (1997) Age differences in implicit learning of higher order dependencies in serial patterns. Psychology and Aging 12(4):634–656.

Howard DV, Howard Jr JH, Japikse K, DiYanni C, Thompson A, Somberg R (2004) Implicit sequence learning: effects of level of structure, adult age, and extended practice. Psychology and aging 19(1):79-92.

Janacsek K, Borbély-Ipkovich E, Nemeth D, Gonda X (2018) How can the depressed mind extract and remember predictive relationships of the environment? Evidence from implicit probabilistic sequence learning. Progress in Neuro-Psychopharmacology and Biological Psychiatry 81:17-24.

Joëls M, Fernandez G, Roozendaal B (2011) Stress and emotional memory: a matter of timing. Trends in cognitive sciences 15(6):280-288.

Kirschbaum C, Hellhammer DH (1994) Salivary cortisol in psychoneuroendocrine research: Recent developments and applications. Psychoneuroendocrinology 19:313–333.

Kirschbaum C, Wolf OT, May M, Wippich W, Hellhammer DH (1996) Stress-and treatment-induced elevations of cortisol levels associated with impaired declarative memory in healthy adults. Life sciences 58(17):1475-1483.

Kóbor A, Janacsek K, Takács Á, Nemeth D (2017) Statistical learning leads to persistent memory: Evidence for one-year consolidation. Scientific reports 7(1):760.

Kóbor A, Takács A, Kardos Zs, Janacsek K, Horváth K, Csépe V, Németh D (2018) ERPs differentiate the sensitivity to statistical probabilities and the learning of sequential structures during procedural learning. Biological Psychology 135:180-193.

Lovallo WR, Farag NH, Vincent AS, Thomas TL, Wilson MF (2006) Cortisol responses to mental stress, exercise, and meals following caffeine intake in men and women. Pharmacology Biochemistry and Behavior 83:441–447.

Lupien SJ, Maheu F, Tu M, Fiocco A, Schramek TE (2007) The effects of stress and stress hormones on human cognition: Implications for the field of brain and cognition. Brain and Cognition 65:209–237.

Maheu M, Meyniel M, Dehaene S (2020) Rational arbitration between statistics and rules in human sequence learning. bioRxiv 2020.02.06.937706

Nater UM, Rohleder N, Gaab J, Berger S, Jud A, Kirschbaum C, Ehlert U (2005) Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. International Journal of Psychophysiology 55(3):333-342.

Németh D, Janacsek K, Fiser J (2013) Age-dependent and coordinated shift in performance between implicit and explicit skill learning. Frontiers in Computational Neuroscience 7(147):1-13.

Nitsche MA, Schauenburg A, Lang N, Liebetanz D, Exner C, Paulus W, Tergau F (2003) Facilitation of implicit motor learning by weak transcranial direct current stimulation of the primary motor cortex in the human. Journal of Cognitive Neuroscience 15(4):619-626.

Roemer S, Schulz A, Richter S, Schachinger H (2009) Oral cortisol improves implicit sequence learning. In Front. Behav. Neurosci. Conference Abstract: 41st European Brain and Behaviour Society Meeting. doi: 10.3389/conf. neuro (Vol. 8, No. 09.280)

Roemer S, Schulz A, Richter S, Lass-Hennemann J, Schächinger H (2011) Oral cortisol impairs implicit sequence learning. Psychopharmacology 215(1):33-40.

Roozendaal B, Okuda S, Van der Zee EA, McGaugh JL (2006) Glucocorticoid enhancement of memory requires arousal-induced noradrenergic activation in the basolateral amygdala. Proceedings of the National Academy of Sciences 103(17):6741-6746.

Schwabe L, Wolf OT (2010) Socially evaluated cold pressor stress after instrumental learning favors habits over goal-directed action. Psychoneuroendocrinology 35(7):977-986.

Schwabe L, Wolf OT (2011) Stress-induced modulation of instrumental behavior: from goal-directed to habitual control of action. Behavioural brain research 219(2):321-328.

Schwabe L, Wolf OT (2012) Stress modulates the engagement of multiple memory systems in classification learning. J. Neurosci. 32:11042–11049.

Schwabe L, Wolf OT (2013) Stress and multiple memory systems: from 'thinking' to 'doing'. Trends in cognitive sciences 17(2):60-68.

Schwabe L, Oitzl MS, Philippsen C, Richter S, Bohringer A, Wippich W, Schachinger H (2007) Stress modulates the use of spatial versus stimulus-response learning strategies in humans. Learning & memory 14(1-2):109-116.

Schwabe L, Haddad L, Schachinger H (2008) HPA axis activation by a socially evaluated cold-pressor test. Psychoneuroendocrinology 33:890–895.

Schwabe L, Römer S, Richter S, Dockendorf S, Bilak B, Schächinger H (2009) Stress effects on declarative memory retrieval are blocked by a β-adrenoceptor antagonist in humans. Psychoneuroendocrinology 34(3):446-454.

Schwabe L, Tegenthoff M, Hoffken O, Wolf OT (2012) Simultaneous glucocorticoid and noradrenergic activity disrupts the neural basis of goal-directed action in the human brain. J. Neurosci 32:10146–55

Seger CA (2018) Corticostriatal foundations of habits. Current opinion in behavioral sciences 20:153-160.

Simor P, Zavecz Zs, Horváth K, Éltető N, Török Cs, Pesthy O, Gombos F, Janacsek K, Nemeth D (2019) Deconstructing procedural memory: different learning trajectories and consolidation of sequence and statistical learning. Frontiers in Psychology 9:2708.

Squire LR, Knowlton B, Musen G (1993) The structure and organization of memory. Annual review of psychology 44(1):453-495.

Szőllősi Á, Keresztes A, Novák B, Szászi B, Kéri S, Racsmány M (2017) The testing effect is preserved in stressful final testing environment. Applied Cognitive Psychology 31(6):615-622.

Takács Á, Kóbor A, Chezan J, Éltető N, Tárnok Z, Nemeth D, Ullman MT, Janacsek K (2018) Is procedural memory enhanced in Tourette syndrome? Evidence from a sequence learning task. Cortex 100:84-94.

Tóth-Fáber E, Tárnok Zs, Janacsek K, Kóbor A, Nagy P, Farkas BCs, Oláh Sz, Merkl D, Hegedűs O, Németh, Takács Á. Dissociation within the procedural system in Tourette syndrome: Enhanced statistical and impaired sequence learning. In preparation.

Ullman MT, Earle FS, Walenski M, Janacsek K (2020) The neurocognition of developmental disorders of language. Annual review of psychology 71:389-417.

Wirz L, Bogdanov M, Schwabe L (2018) Habits under stress: mechanistic insights across different types of learning. Current opinion in behavioral sciences 20:9-16.

Wood W, Rünger D (2016) Psychology of habit. Annual review of psychology 67:289-314.