Abstract

Procedural learning is a vital brain function that allows us to acquire motor skills during development or re-learn them after lesions affecting the motor system. Procedural learning can be improved by feedback of different valence, e.g., monetary or social, mediated by dopaminergic circuits. While processing motivationally relevant stimuli, dopamine interacts closely with oxytocin, whose effects on procedural learning, particularly feedback-based approaches, remain poorly understood. In a randomized, double-blind, placebo-controlled trial, we investigated whether oxytocin modulates the differential effects of monetary and social feedback on procedural learning. Sixty-one healthy male participants were randomized to receive a placebo or oxytocin intranasally. The participants then performed a modified serial reaction time task. Oxytocin plasma concentrations were measured before and after applying the placebo or verum. Groups did not differ regarding general reaction times or measures of procedural learning. For the placebo group, monetary feedback improved procedural learning compared to a neutral control condition. In contrast, the oxytocin group did not show a differential effect of monetary or social feedback despite a significant increase in oxytocin plasma levels after intranasal application. The data suggest that oxytocin does not influence procedural learning *per se*. Instead, oxytocin seems to attenuate the effects of monetary feedback on procedural learning specifically.

Keywords

Motor learning; Motor skill acquisition; Serial reaction time task; Reward learning

1. Introduction

Procedural learning, i.e., acquiring motor skills by practice, is an essential brain function. During childhood and youth, procedural learning is pivotal for acquiring basic motor skills like walking and eating and more complex skills like playing an instrument. Later in life, when motor functions become impaired due to aging-associated and other causes, e.g., after a stroke, re-learning lost or training new motor skills is critical for securing autonomy in activities of daily living.

There is accumulating evidence on the influence of feedback, more specifically reward and punishment, on procedural learning (Galea et al., 2015). Particularly, monetary rewards can improve procedural learning (Wächter et al., 2009) and retention of a motor memory over time (Abe et al., 2011; Galea et al., 2015). Monetary rewards can even help overcome impairments in procedural learning externally induced by inhibitory non-invasive brain stimulation (Wilkinson et al., 2015). Translating these findings to clinical practice, monetary reward (and punishment) enhanced motor adaptation also in stroke patients (Quattrocchi et al., 2017). However, in everyday life and the clinical setting, social reward (e.g., praising children when learning to ride a bike or patients during their interaction with a physiotherapist) is a more common way to foster procedural learning. It was shown that social reward could also enhance procedural learning and retention (Sugawara et al., 2012). For most studies on procedural learning, monetary or social reward was applied. However, the effects of feedback *per se* on procedural learning, as opposed to potential effects of one specific kind or valence of feedback, can only be deduced from studies combining social and monetary feedback and using neutral feedback as a control condition (Doppler et al., 2019).

Oxytocin (OT) is a neuropeptide paramount for social behavior (Macdonald and Macdonald, 2010). After initially being thought to mainly elicit prosocial behavior, more recent studies have shown that OT effects are also context-dependent (Bartz et al., 2011). OT has been shown to facilitate (Gozzi et al., 2017; Hu et al., 2015; Hurlemann et al., 2010; Zhuang et al., 2021) and impair learning with positive social feedback (Clark-Elford et al., 2014). Besides, OT improves the recognition of positive facial expressions (Marsh et al., 2010). Whether OT also modulates or even enhances feedback-based procedural learning is currently unknown. If this is the case, the application of OT in the context of motor rehabilitation could be a promising approach.

Interestingly, OT interacts with the dopaminergic system (Love, 2014), which primarily mediates feedback-based procedural learning (Hosp et al., 2011). This interaction seems to function in both directions: animal studies show that oxytocinergic neurons express dopamine receptors (Baskerville et al., 2009), but also that the application of OT increases the activity of dopaminergic neurons in the VTA (Hung et al., 2017; Xiao et al., 2017). In humans, OT increased the activity of the ventral tegmental area (VTA) (Groppe et al., 2013). VTA neurons project to striatal areas and thereby modulate feedback-based learning. Thus, it can be hypothesized that the application of OT might enhance feedback-based learning and that projections from oxytocinergic neurons to midbrain dopaminergic neurons might represent the underlying structural correlate.

To the best of our knowledge, there are no studies directly investigating the OT effects on procedural learning in general and particularly on feedback-based procedural learning. We, therefore, investigated the differential effects of intranasal OT on feedback-based procedural learning using a modified Serial Reaction Time (SRT) task with monetary, social, and neutral feedback in a randomized, double-blind, placebo-controlled study. We hypothesized a differential effect of monetary and social feedback on procedural learning. More specifically, we expected OT to enhance procedural learning with positive social feedback by OT.

2. Material and methods

2.1. Participants

Sixty-nine healthy male participants aged between 18 and 35 years were randomized to either the placebo or OT group. Exclusion criteria comprised any history of a neurological or psychiatric disorder, the use of psychopharmacologically active medication, and smoking. No women were included to avoid changes in plasma OT related to the menstrual cycle. Subjects were tested for clinically relevant symptoms of depression using the Beck Depression Inventory (BDI) and for hand dominance using the Edinburgh Handedness Inventory. Eight subjects had to be excluded for various reasons (BDI score above the cut-off, $n = 3$; technical problems, $n = 3$; divergence from task instructions, $n = 1$; withdrawal from the study, $n = 1$). Sixty-one participants entered the final analyses, with 29 participants receiving a placebo and 32 receiving OT. The supplement provides the demographic information of the final sample.

We obtained written informed consent from all participants. The ethics committee of the Faculty of Medicine, University of Cologne, had approved the study, which followed the Declaration of Helsinki.

2.2. Procedure

Nasal sprays were manufactured by the pharmacy of the University Hospital Heidelberg and contained either a placebo or 24 I.U. OT. There is evidence that OT nasal spray with this dose leads to elevated OT levels in the cerebrospinal fluid and the blood (Born et al., 2002; Striepens et al., 2013). The external contractor also performed randomization and packaging of the nasal sprays, administered in a double-blind setting. The randomization list was unblinded for data analysis only after the end of data acquisition. Details about randomization and ingredients of the sprays are described in the supplement.

Participants filled out questionnaires (BDI, Edinburgh Handedness Inventory) after providing written informed consent. We also analyzed OT blood levels to verify the correct application of the nasal spray in our sample. Two blood samples were drawn from the participants: the first before applying the nasal spray (baseline), and the second 10 minutes after its application to measure OT plasma concentrations before task initiation. The application of the nasal spray followed published guidelines (Guastella et al., 2013). The behavioral task was started 45 minutes after applying the nasal spray to achieve maximal OT bioavailability in the central nervous system (Striepens et al., 2013).

2.3. Modified Serial Reaction Time Task

We used a modified Serial Reaction Time (SRT) task (Nissen and Bullemer, 1987) (Fig. 1). The task was performed using a standard notebook with a 14” TFT screen (viewing distance: 70 cm) running Presentation (Neurobehavioral Systems) connected to a custom-made three-button keyboard. During the task, a cross appeared in one of three horizontally arranged grey boxes at a time. Subjects were instructed to press the button that spatially corresponded to the location of the cross as fast as possible with their dominant hand. Undisclosed to the subjects, the location of the stimulus either followed a predetermined sequence (S) or a pseudo-randomized order (R). One of three different optimized 9-element sequences was used for each feedback condition. The

sequences were constructed according to specific rules applied in previous studies. These rules enabled avoiding a bias due to potential differences in sequence structures, making the frequency of all stimulus locations and the transition probabilities between consecutive stimulus locations equally distributed across sequences (see Doppler et al., 2019; Dovern et al., 2011). A practice session comprising 45 trials was performed to familiarize the participants with the task and thus minimize a potential bias of general learning effects on response times.

Compared to standard versions, the most significant modification of the current SRT was that we provided performance-dependent feedback after each trial and combined feedback with different valence. Every time the subjects pressed the correct button faster than the criterion RT, this feedback was provided. The criterion was defined as the median RT from the previous experimental block. The median RT from the practice session (one per participant) was used as criterion RT for the first experimental block (R1), the median RT from R1 was used as the criterion for the next experimental block (S2), and so on. We chose to use the median to reduce the impact of potential RT outliers. The duration of the feedback and the inter-stimulus interval was 500 ms. Subjects were given monetary, social, or neutral feedback in three consecutive task runs. A 50 € banknote represented the monetary feedback. Participants had been informed beforehand that every time this stimulus was presented, their compensatory pay would increase by 0.05 €. Social feedback was expressed using an investigator's photo, who was taking care of the respective subject (CEJD, LM, or AS), showing positive feedback ("thumbs up", Fig. 1). A scrambled image of a combination of the stimuli from the monetary and social feedback conditions served as the stimulus for neutral feedback (Fig. 1).

Every participant was exposed to each of the three feedback conditions. Each feedback condition contained 11 experimental blocks: R1-S2-S3-S4-S5-R6-S7-s8-s9-r10-s11. Each block contained five repetitions of one of the three different 9-element sequences. The respective feedback was presented during the first seven blocks (R1-S7). The last four blocks (s8-s11) were performed without feedback to control for a potential distractive effect of the feedback stimuli. The order of feedback conditions as well as the combination of feedback conditions and the 9-element sequences were pseudo-randomized.

3. Results

Participants in the placebo and the OT group did not differ regarding age, handedness, and symptoms of depression (see table in the supplement). The amount of monetary reward (Placebo: 10.9 ± 1.1 €, OT: 10.9 ± 0.9 €, $t(55.5) = 0.96$, $d = 0.01$, $p = 0.96$) and the duration of the experiment (Placebo: 31.5 ± 1 min, OT: 31.8 ± 1.2 min, $t(179) = 1.87$, $d = 0.28$, $p = 0.06$) did not differ between groups.

Mean reaction times (RT) did not differ significantly between medication conditions (Placebo: 320.2 ± 65.5 ms, OT: 340.9 ± 74.3 ms; $F(1,59.29) = 1.78$, $p = 0.19$, $\eta_p^2 = 0.03$), but there were significant differences between feedback conditions (monetary: 323.0 ± 67.6 ms, neutral: 334.6 ± 68.7 ms, social: 335.5 ± 75.9 ms; $F(2,1942) = 15.97$, $p < 0.0001$, $\eta_p^2 = 0.02$) and sequence conditions (predetermined sequence, s/S in Fig. 2: 326.3 ± 72.9 ms, pseudo-randomized order, r/R in Fig. 2: 343.7 ± 64.1 ms; $F(1,1942) = 99.92$, $p < 0.0001$, $\eta_p^2 = 0.05$). Error rates were comparable between groups (Placebo: 4.4 ± 4.7 %, OT: 3.7 ± 4.3 %; $F(1,59) = 0.35$, $p = 0.55$, $\eta_p^2 = 0.006$) and feedback conditions (monetary: 4.0 ± 4.0 %, neutral: 4.1 ± 4.7 %, social: 4.0 ± 4.8 %; $F(2,118) = 0.10$, $p = 0.90$, $\eta_p^2 = 0.002$).

Procedural learning (operationalized by the sequence-specific learning effect, the difference in RT between sequence and random blocks, i.e., the larger the RT difference, the larger the sequence-specific learning effect, see supplement for details) was similar between groups ($F(1,59) = 0.002$, $p = 0.96$, $\eta_p^2 = 0.00004$). However, there was a significant difference in procedural learning between the feedback conditions ($F(2,301) = 4.80$, $p < 0.01$, $\eta_p^2 = 0.03$; monetary: 26.8 ± 51.0 ms, neutral: 17.4 ± 37.3 ms, social: 15.0 ± 42.3 ms). *Post-hoc* tests revealed better procedural learning for monetary compared to social feedback ($t(301) = 2.94$, $d = 0.4$, $p < 0.01$). Notably, there was a significant interaction between the factors "group" and "feedback condition" ($F(2,301) = 4.80$, $p < 0.05$, $\eta_p^2 = 0.03$; see Fig. 3). This interaction was driven by a differential effect of monetary feedback on procedural learning in the placebo group (better procedural learning for monetary feedback when compared to neutral ($t(301) = 2.70$, $d = 0.5$, $p < 0.05$) and social feedback ($t(301) = 3.75$, $d = 0.2$, $p < 0.001$)). In contrast, there was no differential modulation of procedural learning by feedback in the OT group.

For the placebo group only, there was a significant linear trend (β = -11.4, $t(143)$ = -3.52, p < 0.001), indicating a decrease in procedural learning from the monetary to the neutral and social feedback conditions.

The change of plasma OT concentrations by the application of the nasal spray was significantly higher for the OT compared to the placebo group (15.4 ± 11.4 pg/ml versus -1.3 ± 4.6 pg/ml; $t(42)$ = 7.5, d = 1.9, p < 0.0001). However, the plasma OT changes did not correlate with any procedural learning parameter (i.e., sequence-specific learning effect) of the feedback conditions (all p > 0.05, all |r| < 0.2).

4. Discussion

We assessed how OT modulates the effects of monetary and social feedback on procedural learning in a randomized, double-blind, placebo-controlled trial with sixty-one healthy male participants. Feedback-based procedural learning heavily depends on the dopaminergic system (Hosp et al., 2011). Recent evidence shows that OT interacts closely with the dopaminergic system (see review by Love, 2014). Moreover, OT receptors can be found throughout core regions of the motor system, like the basal ganglia. Consequently, OT enhances the activity of midbrain dopaminergic neurons (Xiao et al., 2017) and increases cortico-striatal functional connectivity (Zhao et al., 2019). However, the potential OT effects on procedural learning remain to be elucidated.

Therefore, the current study focused on modulating feedback-based procedural learning by OT. Confirming previous findings (Doppler et al., 2019; Wächter et al., 2009), monetary feedback led to faster reaction times. Notably, the application of intranasal OT did not change reaction times or error rates in general but specifically for the different feedback conditions. This finding suggests that increased plasma OT concentrations do not affect feedback-independent motor performance and procedural learning, which is in line with studies showing no effect of OT on memory in general (Fehm-Wolfsdorf et al., 1988; Ferrier et al., 1980).

In stark contrast to similar procedural learning in both groups in general, the effects of monetary feedback on measures of procedural learning in the placebo group could not be detected after the application of intranasal OT. The observation that intranasal OT does not generally affect procedural learning but affects feedback effects on procedural learning is consistent with the notion

that OT modulates dopaminergic signaling in (feedback-based) procedural learning. An fMRI study (Nawijn et al., 2016) showed that OT led to increased activation in core regions of the brain's reward system during the anticipation of monetary feedback. This could be interpreted as contradicting our data. However, the monetary incentive delay task measures motivational processing, a cognitive process that should be separated from feedback-based procedural learning as measured with the modified SRT in our study. Notably, activation of these brain areas was not associated with changes in motor performance, whereas we found that OT led to impaired motor performance. This might also indicate that different cognitive processes have been investigated. The mechanism behind the effect of OT on procedural learning with monetary feedback warrants further investigation.

Interestingly, intranasal OT did not significantly increase procedural learning under social feedback (placebo group: 9.8 ± 22.0 ms; OT group: 19.7 ± 54.2 ms). However, in contrast to placebo, procedural learning during the social feedback condition did not differ from the monetary feedback condition in the OT group. Therefore, only the placebo group showed a significant linear trend of increasing procedural learning from social over neutral to monetary feedback. This trend was absent in the OT group. This finding contrasts our expectations, given that OT has been shown to improve learning with social feedback (Gozzi et al., 2017; Hu et al., 2015; Hurlemann et al., 2010). Notably, there is considerable variability in behavioral effects of OT even in studies investigating similar processes of social cognition (Striepens et al., 2011). Given the context-sensitive effect of OT (Bartz et al., 2011), it can be argued that this might be due to specific features of the tasks used. More specifically, studies on the facilitation of learning with social feedback investigated association learning, which must be distinguished from procedural learning. Another possible explanation is that the ability of OT to enhance the recognition of facial expressions (Marsh et al., 2010), potentially by increasing the salience of social stimuli (Shamay-Tsoory and Abu-Akel, 2016), might not be sufficient to elicit an enhancement of procedural learning with social feedback. Interestingly, in the study by Marsh et al., changes in reaction times could not be detected either.

A limitation of our study is that we cannot rule out a potential ceiling effect caused by monetary feedback. The quickest reaction times could be detected for both groups for the monetary feedback condition. However, the error rates did not differ between feedback conditions. In case of a ceiling

effect with response times close to the participants' best performance, one might expect a relevant speed-accuracy trade-off, for which there was no indication in our sample. Additionally, it cannot be ruled out that the subjective reward of monetary and social feedback differed between groups. Still, we do not expect this to be the case, as these specific feedbacks did not lead to differences in procedural learning in an earlier study with the same task (Doppler et al., 2019).

Thus, studies investigating the differential effects of different feedback types on procedural learning after OT application are warranted.

5. Conclusions

Even though oxytocin interacts closely with the dopaminergic system, our results show that increased OT plasma levels do not affect procedural learning *per se* but attenuate specifically the effects of monetary feedback. In contrast, OT did not modulate the effects of social feedback on procedural learning.

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Figure legends

Figure 1: Timeline of the modified version of the Serial Reaction Time (SRT) task. Individual reaction time (RT) for the first experimental block had been established in a practice session consisting of 45 random trials before the actual experiment. The median RT from the previous block was used as individual RT in the subsequent experimental blocks. This RT criterion was updated after every further experimental block. Feedback was provided every time the subjects pressed the correct button faster than their individual RT. In three consecutive runs, each subject was given three types of feedback in a pseudo-randomized order: neutral feedback (scrambled image, for control), social feedback (photo of the respective investigator with "thumbs up"), and monetary feedback (a 50 € banknote associated with an increase of the compensatory pay by 0.05 €). The duration of the feedback and the inter-stimulus interval was 500 ms.

Figure 2: Mean reaction times of the modified Serial Reaction Time Task paradigm for both groups and each feedback condition. Mean reaction time of the oxytocin (n = 32) and placebo (n = 29) groups for each feedback condition per block (± SEM). Capital letters identify blocks with feedback, small letters blocks without feedback. S/s identify blocks with a repetitive sequential pattern, R/r blocks with pseudo-random succession. Grey shaded boxes signify blocks used to calculate the sequence-specific learning (SSL) effects.

Figure 3: Procedural learning for each group and feedback condition. Procedural learning is operationalized by the sequence-specific learning effect, calculated by subtracting the mean of the median reaction time of adjacent sequence blocks from the respective random block. Displayed are the means ± SEM (*: $p<0.05$, ***: $p<0.001$).

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GRF serves as an editorial board member of Cortex, Neurological Research and Practice, NeuroImage: Clinical, Zeitschrift für Neuropsychologie, and DGNeurologie; receives royalties from the publication of the books Funktionelle MRT in Psychiatrie und Neurologie, Neurologische Differentialdiagnose, and SOP Neurologie; received honoraria for speaking engagements from Bayer, Desitin, Ergo DKV, Forum für medizinische Fortbildung FomF GmbH, GSK, Medica Academy Messe Düsseldorf, Medicbrain Healthcare, Novartis, Pfizer, and Sportärztebund NRW.

Supplement

Table 1: Demographic characteristics of participants included in the final analysis

	Placebo group (n = 29)	Oxytocin group (n = 32)	*p*
Age [years]	24.6 ± 2.6	25.4 ± 4.0	0.82[1]
Laterality quotient[*]	57.5 ± 61.3	68.5 ± 39.8	0.88[1]
Beck Depression Inventory[**]	2.5 ± 2.3	3.3 ± 2.9	0.29[1]
Change of plasma oxytocin after nasal spray [pg/ml] [***]	-1.3 ± 4.6	15.4 ± 11.4	<0.0001[2]

Values are depicted as mean ± standard deviation.

[1] non-parametric Mann-Whitney-Test, [2] parametric Welch's *t*-test.

[*]Laterality quotient, indicating right-handedness as assessed by the Oldfield Edinburgh handedness inventory (Oldfield, 1971).

[**]Beck Depression Inventory (BDI) (Beck et al., 1961) scores above the cut-off score of 11 indicate clinically relevant depression (Hautzinger et al., 1994)

[***] The change of plasma OT after nasal spray application is denoted as the difference in the OT plasma concentration after and before application of the nasal spray.

Procedure

Randomization was performed using RITA (Randomization In Treatment Arms, Evidat®, version 1.31). A permutated block randomization with a block size of 10 applying a Mersenne Twister algorithm was used. Verum sprays were based on Syntocinon® (Alfasigma S.p.A.). Placebo sprays contained aqua conservans, sodium chloride 0,9%, chlorobutanol, and glycerol.

Blood sampling and analysis

EDTA tubes containing 500 KIU/ml aprotinin were used for collecting blood samples. Blood tubes were centrifuged (15 min at 1600 g) right after the second blood drawing, and plasma aliquots were transferred to -80°C for storage. For analysis, plasma aliquots were thawed, diluted with the same volume of 0.1% trifluoroacetic acid (TFA), and centrifuged (30 min at 18.62g). Solid phase extraction was performed using Sep-Pac Vac 3cc C18 cartridges (Waters, Eschborn, Germany), elution with an acetonitrile-0.1% TFA mixture (volume ratio 95:5), and lyophilization for 21 hours (Vaco2-II; Zirbus, Bad Grunz, Germany). To finally measure OT plasma concentrations, an ELISA kit (Oxytocin ELISA, Enzo Life Sciences, Lörrach, Germany) was used following the manufacturer's instructions. Samples were measured in triplicates with an 8.1 ng/ml lower detection limit. Blood samples of one subject had to be discarded due to an error in pre-analytics.

Statistical analysis

We interrogated the data using RStudio and the packages "lme4" (version 1.1-23) (Bates et al., 2015), "lmerTest" (version 3.1-3) (Kuznetsova et al., 2017), and "emmeans" (version 1.5.3) (Lenth et al., 2021). Group data are presented as mean ± standard deviation unless otherwise stated. The normality of the data was assessed with Shapiro-Wilk tests, Q-Q plots, and density plots.

The median reaction time (RT) per block was calculated to reduce the influence of potential RT outliers in the respective block. We calculated the mean of the median RT of the respective experimental blocks to analyze reaction time differences between medication conditions, feedback conditions and sequence conditions. Following previous studies (Doppler et al., 2019; Dovern et al., 2011; Meier and Cock, 2014), we removed the first trial of each block, error trials, and the trials after error trials to account for post-error slowing, and operationalized procedural learning by the sequence-specific learning effect (SSL), i.e., the difference in median RT between blocks with a predetermined sequence and the surrounding blocks with a pseudo-random succession. In other words, a more considerable difference in median reaction time between blocks with a predetermined sequence and those with a pseudo-random succession leads to a higher sequence-specific learning effect and signifies better procedural learning. The SSL was calculated for blocks

with and without feedback, i.e., the mean of the median RT of the sequence blocks without feedback (s9, s11) was subtracted from the median RT of the random block without feedback (r10). The same operation was performed for the respective blocks with feedback (S5, S7; R6). We decided to only use the sequence blocks mentioned above to calculate SSL (and not S2-S3-S4) to avoid a potential bias by faster reaction times due to generally improved task performance and not due to procedural learning. Block s8 was excluded from the calculation to allow for adaptation to the cessation of feedback after block S7.

Linear mixed models were used to assess error rates, reaction times, and procedural learning. For the linear mixed models using error rates or reaction time as the outcome variable, factors "group" (placebo, OT), "feedback condition" (monetary, neutral, social), and "sequence condition" (predetermined sequence, pseudo-randomized order) as well as their interactions were considered as fixed effects and "subject" as the random effect. To investigate procedural learning, SSL was chosen as the outcome variable, factors "group" (placebo, OT) and "feedback condition" (monetary, neutral, social) as well as their interaction as fixed effects, and "subject" as the random effect. P-values were calculated with a type III ANOVA and Satterthwaite's degrees of freedom method. For post-hoc comparisons, estimated marginal means were calculated using the R package "emmeans" (version 1.5.3) (Lenth et al., 2021). Effect sizes were calculated as partial eta-squared and Cohen's *d*.

In addition, we assessed potential differential effects of procedural learning between feedback conditions by linear trend analysis according to the method proposed by using R packages "lme4" (version 1.1-23) (Bates et al., 2015) and "emmeans" (version 1.5.3) (Lenth et al., 2021).
The change of plasma OT after nasal spray application was operationalized as the difference in the OT plasma concentration after and before application of the nasal spray.

As appropriate, further group comparisons were performed using Welch's *t*-tests, Mann-Whitney tests, and chi-square tests. Correlations were computed with Pearson's correlation coefficient. Significance was accepted at $p<0.05$.

Data availability

Data are available from the first author upon reasonable request. The analytic code is available via GitLab (https://jugit.fz-juelich.de/c.doppler/srt-oxy).

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