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**Heart rate variability synchronization between horses and patients increases across
repeated sessions of horse-assisted therapy**

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12 **Abstract**

13 Physiological synchrony between interacting individuals is thought to support emotional
14 regulation and therapeutic change, but whether such coupling extends across species remains
15 unclear. We investigated heart rate variability (HRV) synchronization, via cross-wavelet power,
16 between therapy horses and female psychiatric patients ($n = 32$) across four standardized horse-
17 assisted therapy sessions, compared with a healthy female control group ($n = 28$) tested once.
18 Using repeated-measures multilevel models, HRV synchronization between horses and patients
19 increased significantly across sessions, exceeding control-group levels by session four despite
20 matching it at session one. During passive, close-contact phases, synchronization was
21 significantly stronger in high- than in low-frequency HRV bands, consistent with shared
22 parasympathetic activity. Patients also reported reduced stress and symptom severity after each
23 session. These findings indicate that horse-assisted therapy promotes dynamic, context-
24 dependent physiological co-regulation between humans and horses, with the therapy horse
25 potentially serving as a non-verbal, non-judgmental partner that supports parasympathetic
26 synchronization during close physical contact.

27 **Keywords**

28 heart rate variability; physiological synchrony; horse-assisted therapy; cross-wavelet power;
29 human-animal interaction

Physiological synchrony is increasingly recognized as a fundamental mechanism underlying social bonds, emotional attunement, and therapeutic change¹⁻³. In human interactions, coordinated physiological processes such as heart rate variability (HRV), respiration, and neural activity have been linked to empathy, emotional regulation, attachment, and interpersonal connectedness⁴. In psychotherapy research, synchrony has also been proposed as a potential mechanism through which therapeutic relationships exert their effects^{2,5}. While this research has primarily focused on human-human interaction, much less is known about whether comparable synchronization processes can also occur between different species. If physiological synchronization can emerge across species, it would suggest that such processes rely on basic, embodied and non-verbal mechanisms rather than shared language or cognitive complexity, with implications for a better understanding of the therapeutic process.

Horse-assisted therapy and cross-species interaction

Horse-assisted therapy (HAT) offers a unique context for investigating such cross-species synchronization processes. In recent decades, horses have been increasingly integrated into psychotherapeutic interventions targeting emotional regulation, interpersonal functioning, trauma-related disorders, and stress-related psychopathology⁶⁻¹⁴. Unlike many other animal-assisted interventions, HAT combines social interaction, close physical contact, rhythmic movement, and embodied nonverbal communication within a highly dynamic interactive context⁹.

Horses are highly social herd animals that rely on nonverbal communication and a rapid sensitivity to environmental and social cues¹⁵. Humans, too, are fundamentally dependent on social connectedness and interpersonal regulation for their psychological and physiological well-being¹⁶⁻¹⁸. This shared social sensitivity can create conditions under which coordinated physiological states may emerge between humans and horses during social interactions^{19,20}. Importantly, interactions with horses differ significantly from human-to-human encounters as they involve fewer verbal and cognitively mediated processes and may

rely more heavily on implicit, embodied, and sensorimotor forms of communication. For people with psychiatric disorders, these cross-species interactions may therefore offer an alternative pathway to affective and physiological co-regulation ¹⁹⁻²¹.

Mechanisms of horse-assisted therapy

Several mechanisms have been proposed to explain the therapeutic effects of HAT. On a behavioral level, horses provide immediate and nonjudgmental feedback to human emotional states ²² potentially facilitating self-awareness and emotional regulation ²³.

Beyond these behavioral processes, HAT also involves a highly embodied form of interaction characterized by rhythmic movement, tactile stimulation, and close somatic contact between human and horse ⁹.

Horseback riding, in particular, introduces repetitive vestibular, tactile, and proprioceptive stimulation that may directly influence autonomic nervous system (ANS) functioning ^{24,25}. Such rhythmic bodily experiences have been associated with relaxation, perceived safety, and reduced arousal, potentially facilitating parasympathetic regulation and embodied forms of co-regulation ^{2,26}. In addition, the therapeutic setting often involves close physical proximity and sustained bodily interaction with the horse, potentially evoking experiences of safety, trust, and attachment-related regulation ²⁷⁻²⁹.

Together, these findings suggest that the therapeutic effects of HAT may not solely arise from verbally or cognitively mediated therapeutic processes but also from dynamic physiological interactions between human and horse. However, despite increasing evidence for the clinical effectiveness of HAT, the physiological mechanisms underlying these effects remain insufficiently understood.

Physiological synchrony and heart rate variability

One promising candidate mechanism is physiological synchrony of the ANS in the horse-human dyad. Physiological synchrony refers to the dynamic coordination of physiological processes between interacting individuals ³. The relevance of synchrony in

82 therapeutic processes is highlighted by ² In-Sync model, which proposes that synchrony across
83 behavioral, emotional and physiological levels may contribute to the therapeutic alliance and
84 psychotherapy outcome. Although originally developed in the context of human psychotherapy,
85 this framework may be extended to horse-assisted therapy, where therapeutic change unfolds
86 through embodied, nonverbal, and cross-species interaction.

87 Such synchronization has been observed in human dyads across a variety of contexts,
88 including romantic relationships, parent-child interactions, cooperative tasks, and
89 psychotherapy ^{30–33}. Synchronization processes are thought to reflect shared attention,
90 emotional attunement, interpersonal regulation, and coordinated behavioral engagement ^{1,4,34–}
91 ³⁶.

92 HRV represents a particularly informative measure in this context, as it refers to the
93 variation in time intervals between successive heartbeats and reflects the dynamic regulation of
94 the ANS ³⁷. High-frequency HRV (oscillations of 0.15 Hz or above) is commonly interpreted
95 as an indicator of parasympathetic activity, whereas lower-frequency oscillations (below 0.15
96 Hz) are thought to reflect a combination of parasympathetic, sympathetic, and baroreflex
97 mechanisms ^{37–42}. HRV is considered a marker of physiological flexibility and adaptive
98 regulation ^{43–45}, with reduced HRV consistently linked to chronic stress and psychiatric
99 disorders ^{43,46,47}. Importantly, impairments in autonomic regulation appear to extend beyond
100 clinical diagnoses. Early life adversity (ELA), chronic stress exposure, and elevated symptom
101 severity have repeatedly been associated with long-term reductions in HRV and altered stress
102 responsivity ^{26,48–51}. Such findings suggest that psychopathology-related factors may influence
103 not only individual autonomic functioning but potentially also the capacity for physiological
104 synchronization with others - since synchronization processes depend on dynamic autonomic
105 responsiveness, chronic reductions in HRV may limit the range of physiological responses
106 available for coupling with an interaction partner ^{35,52}.

Despite growing evidence for the effectiveness of HAT, most existing studies have examined disorder-specific outcomes, focusing on particular diagnoses such as PTSD^{8,10}, depression and anxiety¹⁴, or autism spectrum disorder^{7,12}. A transdiagnostic perspective, examining mechanisms shared across psychiatric conditions rather than specific to any one diagnosis, remains largely absent from this literature. HRV offers a particularly valuable entry point for such an approach as it has been explicitly proposed as a transdiagnostic marker of psychopathology⁴⁸, as autonomic dysregulation and reduced HRV are observed broadly across psychiatric diagnoses, independent of specific symptom profiles. Investigating HRV synchronization in HAT therefore allows for an examination of a fundamental, disorder-independent regulatory mechanism that may underlie the therapeutic potential of HAT across diverse patient populations.

Like humans, horses exhibit measurable HRV reflecting the dynamic regulation of the ANS, with higher HRV associated with relaxation and adaptive autonomic regulation, and lower HRV linked to stress and arousal⁴⁴. Frequency-domain analyses reveal distinct low- and high-frequency components in equine HRV comparable to those in humans: high-frequency components primarily reflect parasympathetic activity, while low-frequency components reflect a combination of sympathetic and parasympathetic influences^{44,53}. Importantly, HRV can be reliably recorded in horses even during movement⁵³, enabling the investigation of dynamic physiological coupling between humans and horses in naturalistic settings.

Measurement of heart rate variability synchronization

Initial psychophysiological studies in HAT have reported changes in autonomic functioning during human-horse interaction, including reductions in stress-related physiological arousal and increases in HRV⁵⁴⁻⁵⁶. Some studies further suggest the possibility of physiological coupling between horses, therapists, and participants during therapeutic interaction^{52,57}. Together, these findings raise the possibility that HAT may involve forms of cross-species autonomic co-regulation^{54-56,58}.

However, existing research remains limited in several important aspects. First, little is known about how synchrony develops over repeated therapeutic sessions. Second, previous studies rarely investigated which specific therapeutic elements or autonomic processes shape synchronization dynamics, in part because most previous studies employed global or time-averaged physiological measures that cannot capture the temporal structure of such effects^{54-57,59}. Third, the influence of psychopathology-related variables such as stress and symptom severity on cross-species synchrony remains largely unexplored.

Importantly, physiological regulation is inherently dynamic rather than static⁶⁰. However, synchronization processes during HAT are likely to fluctuate continuously across different therapy elements, levels of movement, emotional engagement, and bodily contact. To capture these dynamics more precisely, we applied cross-wavelet power (CWP) analyses, which allow the investigation of synchrony simultaneously across time and frequency domains. Compared to conventional analyses focusing primarily on broad low-frequency (LF) and high-frequency (HF) components, CWP enables a more fine-grained characterization of autonomic synchrony by differentiating multiple frequency bands with higher temporal and frequency-specific resolution³¹. Since distinct frequency bands reflect different autonomic processes, as described above, frequency-specific synchrony analysis can reveal not only whether human and horse are physiologically coupled, but which autonomic processes drive this coupling. This approach may be particularly relevant in HAT, where interactional demands continuously shift between active riding, rhythmic movement, tactile contact, and passive resting phases.

Present study

The present study therefore aimed to investigate HRV synchronization between therapy horses and participants during standardized HAT sessions. The population consisted of women with psychiatric disorders, who each completed four HAT sessions, and healthy controls, who completed a single session. We hypothesized that (1) HRV synchronization between therapy horses and participants in the patient group would increase across the four sessions, reflecting

therapeutic progress, insofar as physiological synchrony in therapeutic relationships has been linked to the development of trust, co-regulation, and therapeutic alliance. Furthermore, we anticipated (2) that the synchronization pattern within the patient group would change over the sessions, gradually approaching that observed in the healthy control group during their single session. Additionally, we examined whether psychopathology-related variables such as perceived symptom severity and perceived stress would be associated with HRV synchronization within the patient group. Therefore, we hypothesized that, within the patient group, greater symptom severity (3) and higher perceived stress (4) prior to each session would be associated with reduced HRV synchronization between therapy horse and participant. In addition to hypothesis testing, an exploratory analysis was conducted to investigate whether synchronization dynamics differ not only across specific therapy elements but also across distinct HRV frequency bands.

Results

Our analyses focused on the extracted CWP values across the four HRV frequency bands, which are comparable to traditional high and low frequency band analysis while providing higher temporal and frequency-specific resolution (see Methods).

Frequency specific synchronization across therapy elements

To investigate whether synchronization dynamics differed across therapy elements and HRV frequency bands, an exploratory interaction model (not preregistered) including the interaction between therapy element and frequency band was estimated. The interaction model provided a significantly better fit to the data than a model including only main effects, $\chi^2(33) = 133.97$, $p < .001$, accompanied by a marked reduction in AIC ($\Delta AIC = 71.91$), although BIC increased substantially ($\Delta BIC = 177.50$), reflecting the considerably larger number of estimated parameters (33 vs. 10). In addition, the interaction model explained a larger proportion of variance at the fixed-effects level (marginal $R^2 = .154$) compared to the main-effects model ($R^2_{\text{marginal}} = .145$), while the overall explained variance including random effects also increased

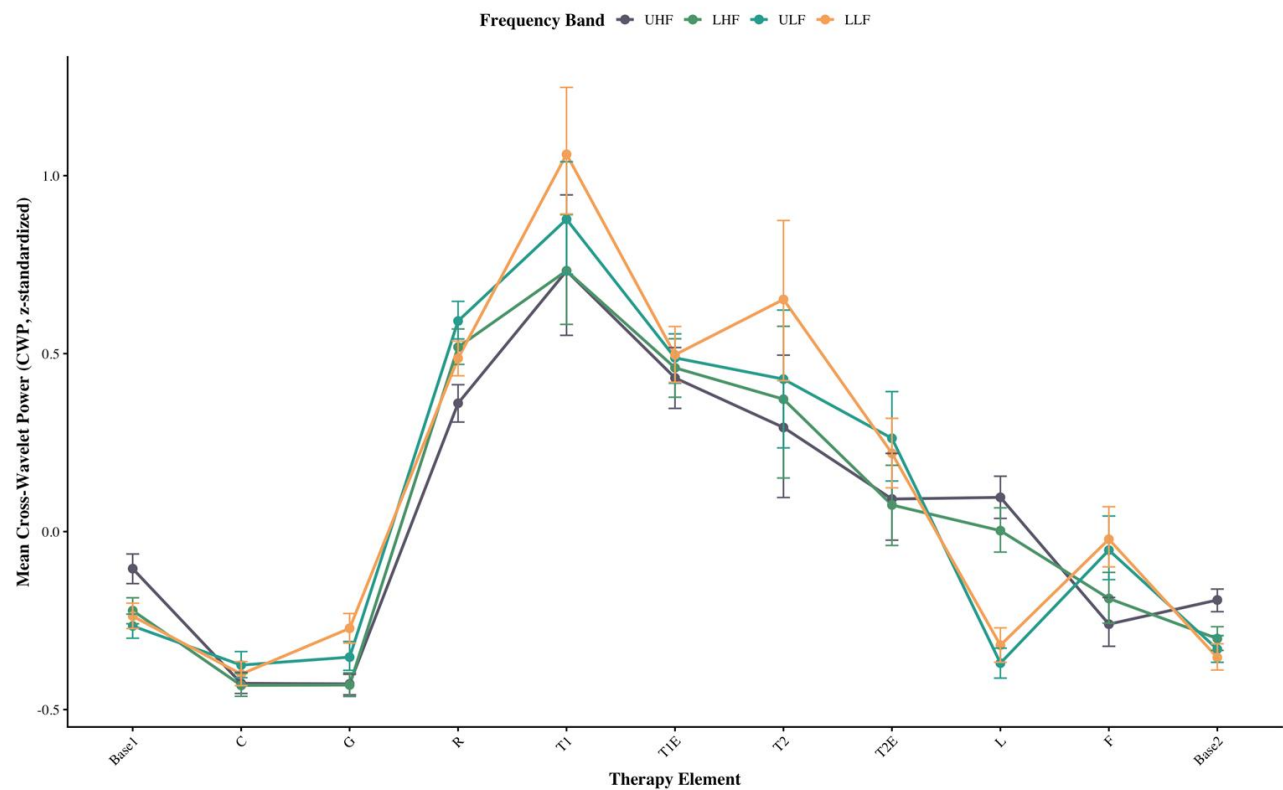
185 slightly ($R^2_{\text{conditional}} = .184$ vs $.176$). Statistical details on model comparisons can be found in
186 the supplementary section.

187 Post-hoc pairwise contrasts between frequency bands were conducted separately for
188 therapy element, with Holm correction applied across the six comparisons within each element.
189 Results showed that synchronization patterns differed substantially across therapy elements and
190 frequency bands. Importantly, frequency-specific differences were not uniformly present across
191 all therapy elements, but emerged particularly during specific interaction phases.

192 During the calmest therapy element, characterized by close physical contact while lying
193 on the horse, pronounced differences between frequency bands were observed. Both high-
194 frequency bands (UHF and LHF) showed significantly higher synchronisation values than both
195 low-frequency bands (UHF > ULF: $p < .001$; UHF > LLF: $p < .001$; LHF > ULF: $p < .001$;
196 LHF > LLF: $p < .001$), while UHF and LHF did not differ from each other ($p = .396$), nor did
197 ULF and LLF ($p = .530$).

198 In contrast, a different synchronization profile emerged during more active phases such
199 as riding. Here, synchronization in the UHF band was significantly lower than in all other
200 frequency bands (LHF: $p < .05$; ULF: $p < .001$; LLF ($p < .001$). Additionally, LHF and ULF
201 differed significantly from each other ($p = .043$), while LHF and LLF ($p = .445$) and ULF and
202 LLF ($p = .183$) did not. Additional frequency-specific differences were observed during selected
203 elements, including grooming or preparation phases, although these effects were less consistent
204 and often did not remain statistically significant following correction for multiple comparisons.

205 **Figure 2. Frequency-Specific HRV Synchrony Across Therapy Elements During HAT**
206 **Sessions**



208

209 *Note.* Points represent mean cross-wavelet power (CWP) values across therapy elements and
210 HRV frequency bands. Error bars represent 95% confidence intervals. Higher values indicate
211 stronger HRV synchrony between participant and therapy horse. Values were z-transformed
212 prior to analysis. Frequency bands included upper-high frequency (UHF), lower-high frequency
213 (LHF), upper-low frequency (ULF), and lower-low frequency (LLF) components of HRV
214 synchrony. Therapy elements comprised baseline phases before and after the session (Base1,
215 Base2), contact/ grooming (C, G), riding-related phases including walking (R), first trot (T1),
216 the continued riding phase directly following the first trot (T1E), second trot (T2), the continued
217 riding phase directly following the second trot (T2E), lying on the horse (L), and farewell (F).

218 ***Does HRV synchronization increase across the four HAT sessions patients?***

219 Hypothesis 1 postulated that HRV synchronization between the therapy horse and the
220 participant within the patient group increases across the four HAT sessions. To test this
221 hypothesis, “session” was integrated into the base multilevel model, which until then included

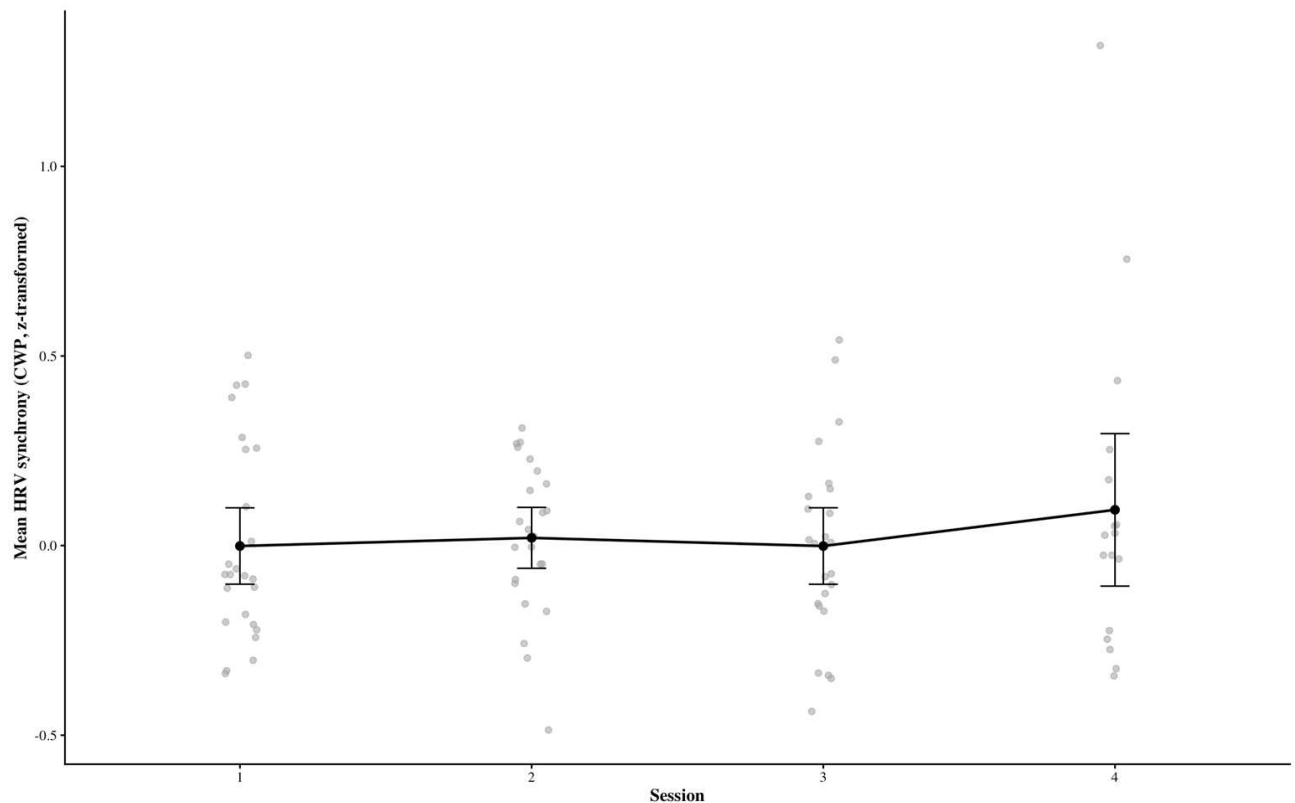
therapy element, frequency band, their interaction, interval, as well as random intercepts for participants and an AR (1) correlation structure to account for repeated measures over time.

Including session as a categorical predictor significantly improved model fit relative to the base model, $\chi^2(3) = 9.75, p = .021$. The session model showed slightly lower BIC values (66923.25 vs. 66944.44), and comparable AIC values (66524.20 vs. 66520.44) compared to the base model. The analysis revealed a significant positive main effect for the fourth session compared to the first session ($b = 0.074, p = .009$).

Given the limited number of sessions ($k = 4$), a linear trend model was estimated as the most parsimonious representation of longitudinal change. Compared to the base model, the linear session model also showed improved fit, $\chi^2(1) = 6.81, p = .009$, accompanied by slightly lower AIC values (66519.39 vs. 66524.20), and revealed a significant positive linear increase in synchronization across sessions ($b = 0.023, p = .009$), as can be seen in Figure 3.

Including session as a predictor did not meaningfully change the proportion of variance explained relative to the model without (marginal $R^2 = .154$ for both models, conditional $R^2 = .184$ for both models), even though the overall model fit improved significantly by likelihood-ratio test. Together, these findings support Hypothesis 1 and indicate increasing HRV synchronization between therapy horses and participants over the course of repeated HAT sessions.

Figure 3. Mean HRV Synchrony Across the Four HAT Sessions



241

242 *Note.* Gray points represent dyad-level mean HRV synchrony values for each session. Black
 243 points and connecting lines represent group means across sessions. Error bars indicate 95%
 244 confidence intervals. HRV synchrony was indexed by z-transformed cross-wavelet power
 245 (CWP), with higher values indicating stronger synchrony between participant and therapy horse.
 246 ***Does the HRV synchronization patterns in the patient group align with those observed in the***
 247 ***control group?***

248 Our second hypothesis proposed that the synchronization patterns in the PG during the
 249 fourth HAT session would more closely resemble those observed in the CG than during the first
 250 session.

251 To test this hypothesis, a linear mixed-effects model was estimated with mean CWP as
 252 the dependent variable. The model included interval, therapy element, frequency, the therapy
 253 element x frequency interaction as fixed effects, with a random intercept for participants and an
 254 AR (1) correlation structure. In accordance with the pre-registered analysis strategy, the

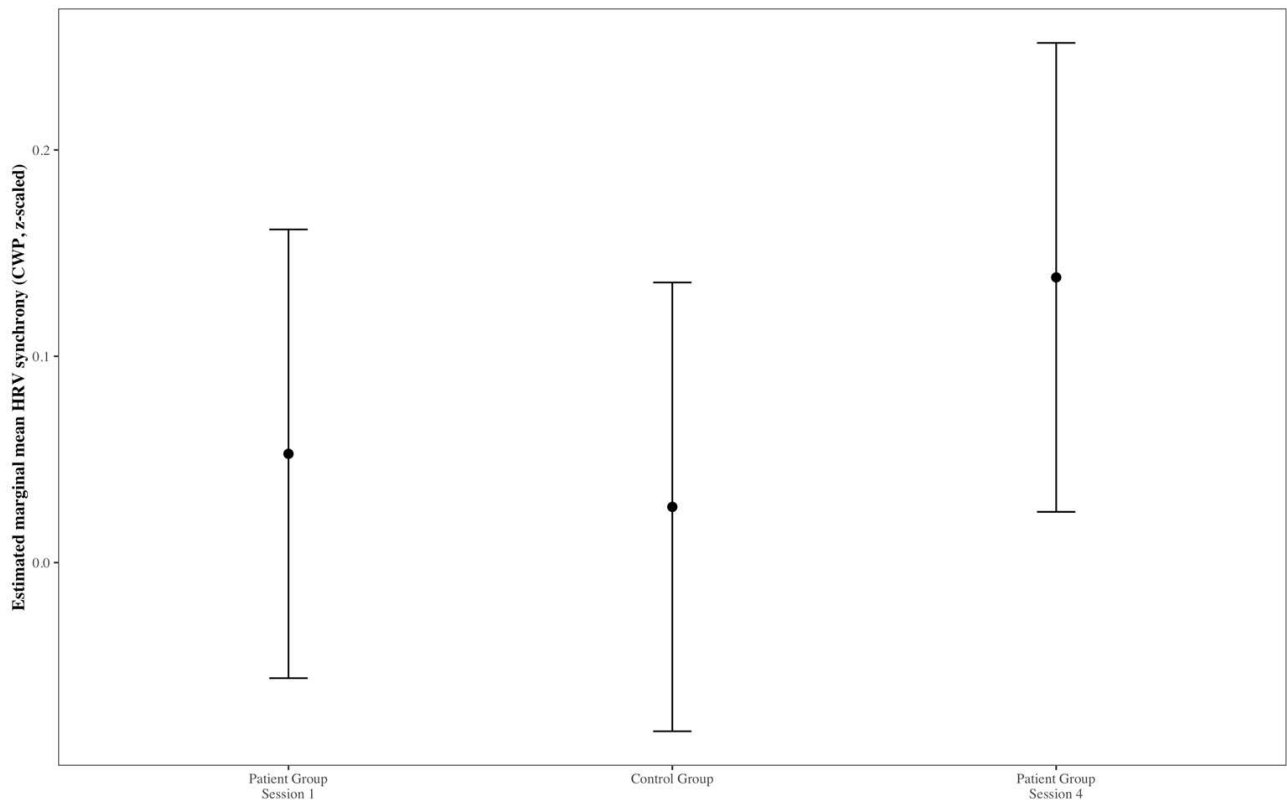
255 predictor variable “group” (consisting of the levels control group [CG], patient group session 1
256 [PG1], and patient group session 4 [PG4]), was added as a predictor.

257 Including group led to a significantly better model fit than the baseline model without
258 this predictor, $\chi^2(2) = 16.38, p < .001$. The group model showed a lower AIC (59032.85 vs
259 59045.22), whereas BIC slightly favored the baseline model (59437.77 vs 59433.95). However,
260 the increase in explained variance was small, with marginal R^2 increasing from .143 to .144 and
261 conditional R^2 from .206 to .208.

262 Post-hoc contrast analyses using Holm correction against the control group revealed no
263 significant difference in HRV synchrony between the patient group in their first session (PG1)
264 and the control group, $b = 0.041, p = .582$. In contrast, HRV synchronization in PG 4 was
265 significantly higher than in the control group, $b = 0.135, p < .001$ (see Figure 4).

266 Although the preregistered statistical criterion was formally met, the conceptual
267 assumption of convergence toward the control group was not supported, as the synchronization
268 trajectories of the patient and control groups diverge rather than converge over time.

269 **Figure 4. HRV Synchrony Across Control and Patient Groups**



270

271 *Note.* Points represent estimated marginal means of HRV synchrony in the control group (CG),
272 patient group during session 1 (PG1), and patient group during session 4 (PG4). Error bars
273 represent 95% confidence intervals. HRV synchrony was indexed using z-standardized cross-
274 wavelet power (CWP), with higher values indicating stronger synchronization between
275 participant and therapy horse.

276 ***Effect from perceived symptom severity on HF-HRV synchronization in the patient group.***

277 Hypothesis 3 assumed an inverse relationship and posited that higher initial symptom
278 severity in patients would predict lower HRV synchronization between therapy horse and
279 participant. The analysis was restricted to the $n = 23$ patients with complete symptom severity
280 data. Comparing the model including symptom severity as a main effect to the base model did
281 not yield a significant improvement in model fit, $\chi^2(1) = 0.91$, $p = .340$, indicating no robust
282 overall association between symptom severity and HRV synchronization. Thus, Hypothesis 3
283 was not supported.

284 To examine whether symptom-related effects varied across therapy contexts and
285 frequency bands, an exploratory interaction model including symptom severity, therapy element,
286 and frequency band was estimated. This interaction model significantly improved model fit,
287 $\chi^2(43) = 132.41$, $p < .001$, and was accompanied by lower AIC values (29636.91 vs. 29683.32).
288 However, BIC increased substantially (30325.53 vs. 30050.08), reflecting the heavy penalty for
289 43 additional parameters. Explained variance increased from $R^2_{\text{marginal}} = .177$ to .191 and
290 $R^2_{\text{conditional}} = .218$ to .232 compared to the base model.

291 Exploratory slope analyses revealed context-dependent associations between symptom
292 severity and HRV synchronization. Significant negative associations emerged during riding (R:
293 UHF $b = -0.070$; LHF $b = -0.102$) and the end of the first trotting phase (T1E: UHF $b = -0.218$;
294 LHF $b = -0.161$), indicating that patients with higher symptom severity showed less HRV
295 synchronization in the higher frequencies during active movement phases. An unexpected
296 positive association emerged during the second trot phase (T2: UHF $b = 0.359$). No significant

297 associations were observed during passive therapy elements or in the low-frequency bands.
298 Given their exploratory nature, these findings require replication.

299 ***Effect from perceived stress on HRV synchronization in the patient group.***

300 Hypothesis 4 postulated a similar inverse relationship regarding acute stress, assuming
301 that higher perceived stress prior to the session would correlate with lower HRV synchrony
302 between participant and therapy horse. The analysis was restricted to the $n = 31$ patients with
303 complete perceived stress data. Comparing the model including perceived stress as a main effect
304 to the base model did not yield a significant improvement in model fit, $\chi^2(1) = 3.22$, $p = .073$
305 (AIC = 60365.47 vs. 60364.25; BIC = 60759.74 vs. 60766.74), indicating no robust overall
306 association between perceived stress and HRV synchronization. Thus, Hypothesis 4 was not
307 supported.

308 To examine whether acute stress-related effects varied across therapy elements and
309 frequency bands, an exploratory interaction was computed. This model significantly improved
310 fit over the main effect model, $\chi^2(43) = 72.48$, $p = .003$. However, AIC (60377.77) and BIC
311 (61133.45) values were both higher than in the base model.

312 Exploratory slope analyses revealed five therapy element x frequency band
313 combinations with significant associations. During the riding element, perceived stress was
314 negatively associated with HRV synchronization in both the UHF ($b = -0.066$) and the LHF (b
315 $= -0.044$) band, indicating that patients with higher pre-session stress showed less physiological
316 coupling with the horse during riding. A similar negative pattern emerged during the end of the
317 first trotting phase in UHF ($b = -0.055$) and during the second trotting phase in the LLF band
318 ($b = -0.118$). In contrast, the end of the second trotting phase in the UHF band showed a
319 significant positive slope ($b = 0.089$), suggesting greater synchronization with higher pre-
320 session stress during this element. No significant associations were found during passive or
321 resting elements. Given the exploratory nature of these analyses, findings should be interpreted
322 with caution and require replication.

323 *Acute changes in perceived stress and symptom severity across HAT sessions*

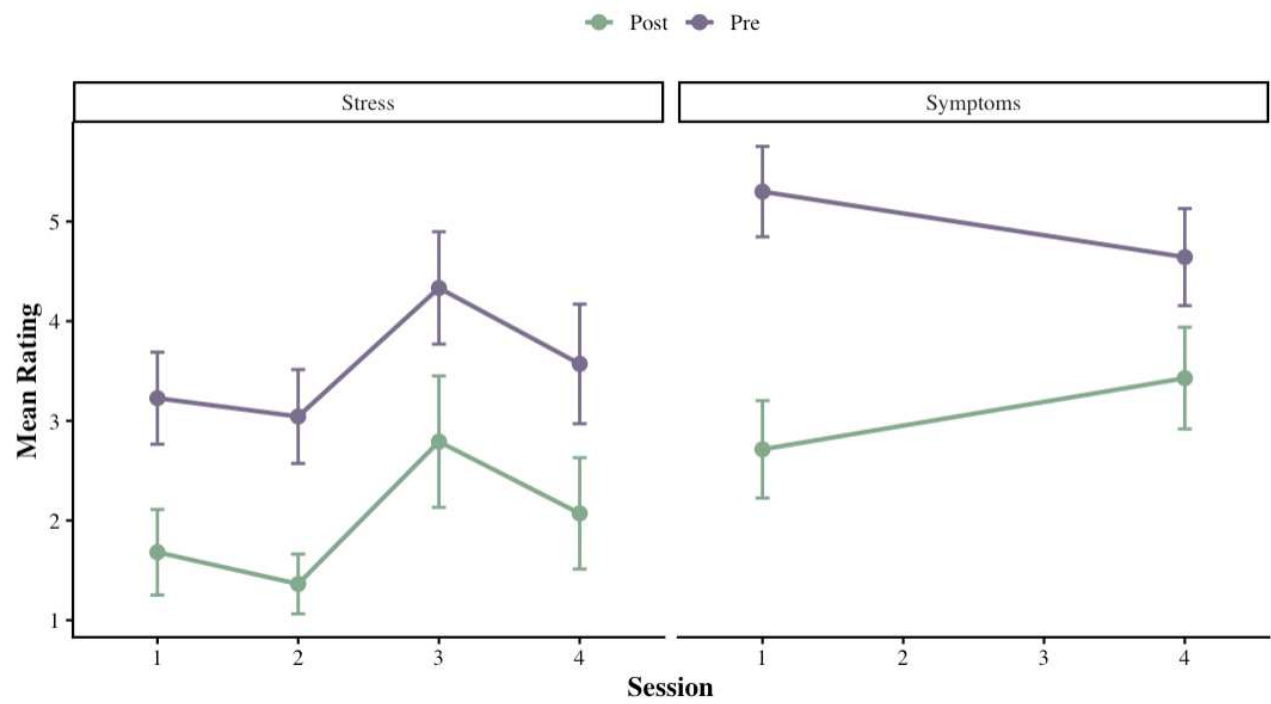
324 To examine whether participants reported acute changes in perceived stress and
325 symptom severity across HAT sessions, additional exploratory linear mixed-effects models with
326 random intercepts for participants were estimated. Each participant contributed two
327 observations per session, one pre- and one post-session measurement, with timepoint (pre vs.
328 post) as a fixed effect, treating repeated measurements as nested within sessions and sessions
329 nested within participants. Session was subsequently added as a predictor to examine whether
330 the magnitude of pre-to-post change differed across the four HAT sessions.

331 For acute perceived stress ($n = 31$), the base model (with timepoint only) showed a
332 significant reduction from pre- to post-session, $b = 1.58$, $t(130.86) = 5.20$, $p < .001$, indicating
333 lower stress ratings following the HAT sessions. Adding session as an additional predictor did
334 not significantly improved model fit, $\chi^2(1) = 3.01$, $p = .083$, and the timepoint x session also
335 did not improve fit, $\chi^2(1) = 0.01$, $p = .930$. These findings indicate that perceived stress
336 decreased consistently from pre- to post-session across all HAT sessions, with no evidence for
337 systematic variation in this effect across sessions.

338 Similarly, for symptom severity ($n = 29$), the base model revealed a significant pre-to-
339 post reduction, $b = 1.45$, $t(86.62) = 4.04$, $p < .001$. Including session as an additional predictor
340 did not result in improved model fit, $\chi^2(1) = 0.22$, $p = .640$, nor did the timepoint x session
341 interaction, $\chi^2(1) = 2.79$, $p = .096$. Together, these findings suggest that symptom severity
342 decreased reliably following each HAT session, with no evidence that the magnitude of
343 reduction differed systematically across sessions.

344 **Figure 5. Stress and symptom severity across HAT sessions.**

345



346

347 *Note.* Mean perceived stress and symptom severity ratings before (Pre) and after (Post) each
348 horse-assisted therapy (HAT) session. Points represent observed means across participants, and
349 error bars indicate ± 1 standard error. Perceived stress was assessed before and after each of the
350 four sessions; symptom severity was assessed before and after sessions 1 and 4 only. Across
351 all measured sessions, both perceived stress and symptom severity were lower following the
352 HAT sessions compared to pre-session ratings.

353

Discussion

354 The present study investigated physiological synchrony in HRV between patients and therapy
355 horses as a potential mechanism underlying HAT. The findings provide a nuanced picture of
356 how cross-species physiological coupling emerges over time and is shaped by contextual and
357 individual factors.

358 A central finding of this study is that HRV synchrony was not uniform across the HAT session
359 but varied systematically across therapy elements, indicating a strong context dependency of
360 cross-species physiological coupling. Synchrony increased significantly when participants

361 began riding, suggesting that participation in coordinated, movement-based interaction can
362 facilitate physiological alignment between human and horse. Riding leads to a shared
363 rhythmic structure and continuous sensorimotor coupling, processes known to facilitate
364 interpersonal coordination and physiological alignment ^{2,61–63}. This finding underscores the
365 importance of active participation and embodied interaction for the emergence of synchrony.

366 In contrast, a distinctly different pattern was observed during the lying position,
367 characterized by minimal movement and close physical contact. Here, synchrony exhibited a
368 clear frequency-specific modulation with increased coupling in the higher-frequency bands
369 compared to the lower-frequency bands. Since higher-frequency HRV components are
370 typically associated with parasympathetic and vagal activity and lower-frequency components
371 reflect more complex sympathetic, parasympathetic and baroreflex-related processes ^{37,38,42,64},
372 this pattern suggests that synchrony may be underpinned by different autonomic processes
373 depending on the interaction context. Importantly, synchrony did not increase uniformly
374 across all frequency bands during this phase. The absence of parallel increases in the low-
375 frequency components suggests that synchrony cannot be interpreted as general co-activation
376 or a shared arousal response. Instead, the results point to differentiated physiological
377 processes, whereby passive, low-demand interaction contexts may favor forms of
378 physiological attunement that differ from those observed during active coordination. The
379 selective increase in high-frequency synchrony during the lying position may specifically
380 reflect shared parasympathetic activation, a process linked in individual physiology to
381 emotion regulation, feelings of safety, and social-emotional processing ^{26,65}. This suggests that
382 the activation demands of a given therapy element may shape which autonomic frequency
383 components become entrained between human and horse, with passive, low-demand
384 interactions preferentially engaging parasympathetically mediated attunement. Thus, these
385 passive interaction contexts involving touch, stillness and bodily proximity may facilitate
386 forms of physiological attunement associated with safety and co-regulation ^{26,65,66}.

387 Taken together, these results suggest that synchrony in horse-assisted therapy can arise
388 through multiple pathways. While active phases such as riding appear to promote synchrony
389 through shared movement and rhythmic coordination, passive phases involving stillness and
390 physical contact may support a different form of physiological attunement. This dual pattern
391 underscores the importance of considering both interaction dynamics and the specificity of the
392 autonomic nervous system when interpreting cross-species synchrony.

393 In line with Hypothesis 1, HRV synchrony increased significantly across the four
394 therapy sessions. Both categorical and linear models showed a clear upward trend, suggesting
395 that the physiological synchrony between participants and therapy horses is strengthened
396 through repeated interactions. This pattern is consistent with the notion that synchrony reflects
397 a dynamic process that develops through familiarity, trust, and repeated shared experiences
398 ^{2,65}.

399 Regarding Hypothesis 2, the comparison with the control group revealed a more
400 complex pattern. While patients did not differ significantly from healthy controls in their first
401 session, synchrony in the fourth session exceeded that of the control group. Although the
402 predefined statistical criterion was met, the conceptual assumption of convergence toward a
403 “normative” level of synchrony was thus not confirmed. Instead, over time, the patients
404 developed a stronger physiological coupling with the therapy horse than the control
405 participants. One possible interpretation is that individuals with a greater need for regulation
406 engage more deeply in cross-species synchronization processes once a stable interactive
407 context is established ⁵². Rather than reflecting normalization, the increased synchrony could
408 indicate an adaptive, context-specific integration into the relationship with the therapeutic
409 partner. However, this interpretation should be treated with caution, as the comparison is
410 inherently limited by the asymmetry between groups, whereas patients interacted with the
411 therapy horse across four sessions, control participants attended only one. The elevated
412 synchrony observed in session 4 may therefore partly reflect familiarization and habituation

413 effects from repeated exposure, rather than a specific therapeutic process. Notably, each
414 participant interacted with the same horse and therapist across all four sessions, suggesting
415 that the observed increase in synchrony may reflect the development of a specific dyadic
416 relationship rather than a general familiarity with HAT.

417 This interpretation is supported by findings from ⁵²which demonstrated that
418 individuals with early life adversity show reduced synchrony in human–human interactions,
419 but not in human–horse interactions. Importantly, this suggests that impairments in synchrony
420 may be specific to interpersonal contexts involving social evaluation, cognitive demands, or
421 attachment-related expectations, whereas cross-species interactions may rely more strongly on
422 implicit, embodied, and nonverbal regulatory processes ^{2,26,67,68}. In this framework, the
423 absence of reduced synchrony in human–horse dyads, and the increase observed in the present
424 study, can be understood as reflecting a preserved or even preferentially accessible pathway
425 for physiological attunement. The therapy horse may also serve as an emotional anchor,
426 providing a non-threatening, non-judgmental partner that supports regulation.

427 Contrary to initial expectations, neither symptom severity nor perceived stress showed
428 a robust general association with HRV synchrony. However, both predictors significantly
429 improved model fit when their interactions with therapy elements (and, in some cases, with
430 frequency bands) were taken into account. These interaction effects exhibited a context-
431 dependent pattern as higher symptom severity and higher stress were associated with reduced
432 synchrony during active phases of the intervention, such as riding and trotting. In contrast,
433 these negative associations were not observed during calm phases, such as lying. This
434 suggests that increased psychological stress does not uniformly impair synchrony, but may
435 specifically disrupt physiological coordination under conditions that require heightened
436 cognitive, emotional, or motor engagement, consistent with theories linking chronic stress and
437 psychopathology to altered autonomic flexibility and reduced regulatory capacity ^{26,48}.

438 Conversely, less demanding contexts can provide a regulating environment in which
439 synchrony can emerge even in highly stressed individuals.

440 Several limitations should be considered when interpreting the findings of this study.
441 The study was strictly limited to physically healthy women and female patients between the
442 age of 18-45. Consequently, the observed patterns of human-horse HRV synchronization
443 cannot be automatically generalized to men, children, adolescents or older populations.
444 Furthermore, our patient group included individuals with a highly heterogenous range of
445 psychiatric diagnoses, such as depression, PTSD, borderline personality and anxiety disorder,
446 often accompanied by varying comorbidities. While the diagnostic diversity reflects clinical
447 reality, it limits the ability to determine whether the physiological synchronization process is
448 distinctly influenced by specific psychiatric conditions. Additionally, psychiatric diagnoses
449 were based on participant self-reporting during our screening, as participants indicated
450 whether they were currently in psychotherapeutic treatment and their respective diagnosis. No
451 formal clinical diagnostic assessment was conducted, which may have introduced diagnostic
452 inaccuracies. Furthermore, the analyses of symptom severity and perceived stress as
453 predictors of HRV synchrony were restricted to the patient group, as these variables are not
454 clinically meaningful in healthy controls. Future studies including clinical comparison groups
455 could examine whether these associations are specific to individuals with psychiatric
456 diagnoses. Importantly, the control group participated in only one HAT session, limiting the
457 comparability with patient group session 4. Future studies should include a multi-session
458 control group to disentangle familiarization effects from session-specific therapeutic
459 processes.

460 Also, collecting continuous HRV data from moving animals in a naturalistic therapy
461 setting proved technically demanding and resulted in increased susceptibility to motion
462 artifacts and signal loss, which are common challenges in ambulatory HRV research ^{37,69,70}.
463 This significant data loss led to the exclusion of several participants due to incomplete HRV

464 recordings, and this reduced sample size thus may have limited the statistical power of the
465 models, which could partly explain why the global main effects for perceived stress and
466 symptom severity narrowly missed conventional significance levels across the entire session.
467 At the same time, this naturalistic design reflects the conditions of real-world HAT practice,
468 which can be considered a methodological strength.

469 Complementing the physiological findings, analyses of the pre- and post-
470 measurements showed a significant reduction in both perceived stress and symptom severity
471 following the intervention. Notably, the magnitude of these within-session reductions did not
472 increase systematically across the four sessions, suggesting that HAT may provide consistent
473 immediate relief rather than cumulative psychological improvement within a short
474 intervention window. Whether long HAT programs produce accumulating benefits remains an
475 open question for future research. Although causal conclusions are limited by the naturalistic
476 study design, these results are consistent with previous evidence that HAT can bring about
477 psychological improvement in addition to measurable changes in physiological interaction
478 patterns ^{6,7,10}.

479 Overall, the present findings suggest that physiological synchrony in horse-assisted
480 therapy is a dynamic, context-sensitive process rather than a stable individual characteristic.
481 Synchrony increased across repeated sessions, varied systematically across interaction context
482 and frequency bands, and was differentially influenced by individual characteristics such as
483 stress and symptom burden. Importantly, the results indicate that low-demand interaction
484 phases involving stillness, bodily proximity, and physical anchoring may provide particularly
485 favorable conditions for physiological attunement, consistent with theoretical models
486 emphasizing embodied co-regulation and autonomic safety ^{26,65}. Notably, patients not only
487 matched but ultimately exceeded control levels of synchrony by the fourth session, suggesting
488 a particular affinity for physiological coupling with horses. This raises the possibility that
489 HAT may offer patients an alternative pathway to experience physiological co-regulation, one

490 that may be less accessible in human-human interactions, where psychiatric symptoms can
491 interfere with synchronization processes.

492 More broadly, the results support the view that cross-species physiological synchrony
493 may represent a relevant mechanism underlying animal-assisted intervention. At the same
494 time, the findings emphasize that synchrony should not be conceptualized as a unitary
495 phenomenon, but rather as a dynamic process emerging from the interaction between
496 autonomic regulation, contextual demands, and embodied social engagement^{2,3}. By
497 integrating temporal and frequency-specific analyses of HRV synchrony, the present study
498 contributes to a more differentiated understanding of how physiological co-regulation may
499 unfold in human-animal therapeutic contexts.

500 **Methods**

501 Note that data from the same project has been previously reported in⁵², which examined
502 the influence the relationship between early life adversity and physiological synchrony within
503 the therapeutic triad (participant, horse, and riding therapist), using the same sample and data
504 collection procedure.

505 The data collection relevant to this work included three periods, each covering summer
506 months from April to September of 2019-2021. Both participants and experimenters wore face
507 masks during data collection in the summer of 2021 in adherence to COVID-19 guidelines. All
508 performed procedures followed regulations set forth by the Declaration of Helsinki and were
509 approved by the institutional review board of the University of Konstanz (IRB statement
510 12/2017). We preregistered our study and analyses plans at the Open-Science Framework (⁷¹;
511 <https://doi.org/10.17605/OSF.IO/TZCYQ>).

512 **Deviations from preregistration**

513 Several aspects of the present analysis deviate from the preregistered plan. First,
514 whereas the preregistration specified wavelet coherence as the primary synchronization metrics,
515 we instead used CWP to quantify HRV synchrony. Unlike coherence, which normalizes for

516 signal amplitude and captures phase consistency, CWP retains information about the absolute
517 magnitude of shared spectral power, which we considered more informative for capturing the
518 strength and not just the consistency of physiological coupling.

519 Second, the preregistration specified 10-second intervals nested within one-minute
520 segments as the unit of temporal resolution. The present analyses instead used 30-second
521 intervals, chosen to align with the practical durations of the therapy elements.

522 Third, the final analytic sample ($N = 60$) is smaller than the sample already reported as
523 complete-case in the preregistration ($N = 81$). This additional reduction reflects the stricter
524 continuity requirement of the CWP approach. Because it requires temporally continuous HRV
525 signals from both members of a dyad, any gap exceeding one minute in either partner's
526 recording led to exclusion of the entire dyad, resulting in further data loss beyond what had
527 been anticipated at the time of preregistration.

528 **Sample**

529 Control group participants were recruited via flyers in the university and experiment
530 group participants were recruited in psychiatric institutions in and around the city of Konstanz,
531 Germany.

532 A total of 92 participants took part in the study. Due to exclusion criteria, only physically
533 healthy women with a maximum weight of 80 kg, aged between 18 and 45 years, who did not
534 take drugs and did not consume more than five cigarettes per day, were allowed to participate.
535 The weight limit was imposed for the safety and welfare of the therapy horses; the average
536 range was chosen to minimize age-related variation in HRV; and restrictions on substance use
537 were applied because smoking and drug use are known to substantially alter autonomic nervous
538 system functioning and HRV ^{37,72}.

539 Of the total sample, a substantial portion had to be excluded from the final analytic
540 sample due to incomplete HRV data. Because the CWP approach requires temporally
541 continuous HRV signals from both members of a dyad, any gap exceeding one minute in either

the participant's or the horse's ECG recording during a session led to the exclusion of the entire dyad from analysis. Data loss occurred particularly often in the horse's ECG signals, which were prone to motion artifacts and poor electrode contact caused by the horses' coat and movement during grooming and riding. As a result, the final analytic sample included 60 women aged 18 to 45 years ($M = 26.49$, $SD = 7.06$). Participants' body mass index (BMI) ranged from 14.20 to 32.90 kg/m² ($M = 22.18$, $SD = 3.03$).

The analytic sample consisted of a healthy control group (CG) and a patient group (PG). The CG comprised 28 mentally and physically healthy women aged between 18 and 27 years ($M = 22.50$, $SD = 2.52$). The patient group consisted of 32 women currently undergoing psychotherapeutic treatment, aged between 19 and 45 years ($M = 29.88$, $SD = 7.89$). A comparison of demographic and clinical characteristics across groups is provided in Supplementary Table 1.

Participants in the patient group presented with a variety of psychiatric diagnoses. For descriptive purposes, diagnoses were grouped into three broader categories. The most frequent diagnoses were affective disorders ($n = 20$), primarily depressive disorders, followed by trauma- and anxiety-related disorders ($n = 8$), including PTSD and social anxiety. Additional diagnoses included somatoform and personality disorders ($n = 4$).

Questionnaires, therapy horses and material

In the presurvey, participants completed an online screening questionnaire encompassing inquiries about age, weight, gender, and both mental and physical health. ELA was evaluated using both the Childhood Trauma Questionnaire (CTQ⁷³) and the Parental Bonding Instrument (PBI, ^{74,75}). In line with the preregistered plan, the relationship between ELA and HRV synchronization was examined separately in Wienhold et al. (2025), using the same sample and data collection procedure, thus ELA is not analyzed as a separate predictor in the present study.

567 Eligible participants were subsequently scheduled for the therapy sessions based on
568 availability. Before the actual riding therapy elements, all participants provided informed
569 consent and completed several questionnaires. Acute stress levels were assessed using a 0 - 10
570 visual analog scale. Chronic stress levels were evaluated for the patient group in the second
571 session through the Perceived Stress Scale (PSS⁷⁶), as the first session was used to establish
572 baseline physiological data and familiarize participants with the procedure. This widely used
573 measure captures the degree to which situations in one's life are perceived as stressful over the
574 past month. All participants in the patient group completed the Shutdown Dissociation Scale
575 (Shut-D, ⁷⁷ questionnaire before the third HAT session to systematically evaluate dissociative
576 responses and gain insights into trauma-related symptomatology ⁷⁸. Participants in the patient
577 group also rated the perceived severity of their symptoms, and the extent to which their personal
578 and professional lives were affected on a 0 - 10 VAS at both the first and last sessions. Following
579 each therapy session, participants provided detailed feedback on their overall experiences and
580 the quality of their interactions during the riding therapy session via open-ended written
581 questions.

582 Horse-assisted therapy sessions involved four well-trained therapy horses, which are
583 well-versed in therapy work and exhibit variations in both internal temperament and external
584 characteristics, such as size, breed, and age. Specifically, the equine team included an 8-year-
585 old Connemara and a 13-year-old Fjord gelding (named Forest and Karlson), an 18-year-old
586 Fjord, and a 19-year-old Criollo mare (named Jill and Bonita).

587 During the therapy session, we utilized specialized equipment to ensure safety and
588 comfort of both participants and horses. This included a therapy halter, a lead rope, a therapy
589 pad, and a belt equipped with handholds. Additionally, participants were provided with brushes
590 and helmets.

591 Heart rates of the participant, riding therapist, and therapy horse were tracked using
592 Polar chest straps equipped with H10 sensors ⁷⁹, which were connected via Bluetooth to iPhones
593 and iPads. The HRV Logger App ⁸⁰ was installed on all three devices for data collection.

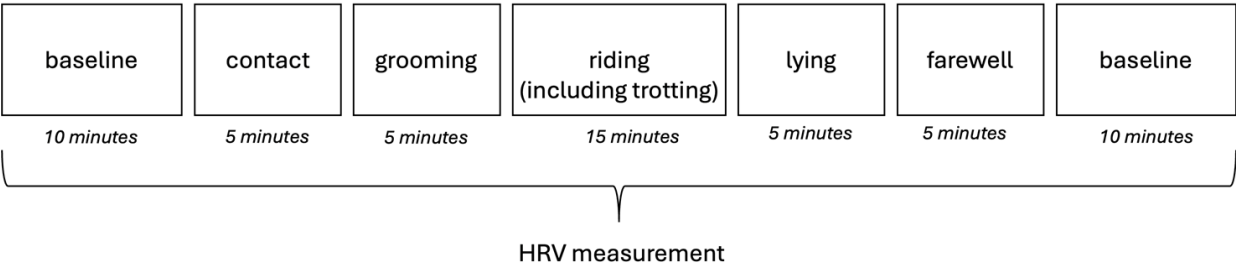
594 **Study design**

595 On the day of testing, sessions were scheduled at either 8:45 a.m. or 10:30 a.m. and
596 lasted 60 minutes, in order to minimize diurnal variation in HRV ³⁷. Control group participants
597 underwent one standardized HAT session (encompassing contact, grooming, riding, lying, and
598 farewell elements; described in detail below), while the patient group participated in four
599 sessions following the same protocol. The single session for the control group served as a
600 normative reference point, while the four sessions for the patient group were designed to
601 approximate the structure of a short-term therapeutic intervention and to capture the
602 development of synchronization across repeated human-horse interactions. Session numbers
603 across HAT programs vary considerably depending on protocol and population, ranging from
604 as few as three to more than twenty sessions, with most falling within a range of four to eight
605 sessions ⁸¹; four sessions were therefore considered sufficient to capture early synchronization
606 dynamics within the constraints of a research design. Each participant was assigned to the same
607 therapy horse and riding therapist across all four sessions to ensure consistency of the dyadic
608 interactions. The four sessions were scheduled at varying intervals, depending on participant
609 availability and organizational constraints, with intervals ranging from a minimum of one day
610 to a maximum of four weeks, and averaging approximately one week.

611 Initially, participants were briefed on the study's objectives and potential risks.
612 Participants then proceeded to complete questionnaires, for about ten minutes. Following this,
613 there was a five-minute contact session with the horse, during which participants interacted
614 with and petted the horse. The riding therapist then guided the participant in grooming the horse
615 for five minutes. The horse was then fitted with a therapy pad and riding belt and led to the
616 riding arena for a fifteen-minute riding session, including two short trotting sequences.

617 Following the riding session, the horse was brought to a halt in the middle of the riding arena,
 618 and the participant was instructed to lie prone on the horse's back for five minutes. Subsequently,
 619 the horse was returned to the therapy box, and the participant was allowed to say goodbye to
 620 the horse before engaging in a questionnaire session lasting an additional ten minutes. Finally,
 621 the HRV chest strap was removed, and participants received €15 as compensation. The
 622 experimental procedure is also visualized in Figure 1.

623 **Figure 1.** Experimental procedure.



624
 625 **Analysis of HRV (synchronization)**
 626 **Data cleaning**

627 **Data processing.** The heart rate data was processed using the statistics program R ⁸² in RStudio
 628 ⁸³ and included searching for and correction of artifacts and interpolating missing values. Since
 629 HRV data from horses present with more artefacts than present in recordings from humans,
 630 artifacts were identified by running cluster analyses over the entire dataset first, followed by
 631 visual inspections to identify the artefact cluster. This cluster was then removed, and missing
 632 values were subsequently interpolated ⁵². The resulting dataset was then entered into the normal
 633 processing pipeline for HRV synchrony analysis, including artifact detection and correction of
 634 implausible outliers using a criterion based on the relative deviation of each R-R interval from
 635 the respective preceding interval, with a threshold starting at $\pm 20\%$ and increased in 5% steps
 636 (up to a maximum of $\pm 50\%$) whenever more than 10% of the total data set was flagged for
 637 correction at the current threshold. If the gap of missing data did not exceed 10 seconds, missing

638 data were interpolated from surrounding R-R intervals; otherwise, the recordings were excluded
639 from analysis. R packages used for analyses included RHRV⁸⁴, parsedate⁸⁵, haven⁸⁶, foreign
640⁸⁷, WaveletComp⁸⁸, dplyr⁸⁹, and lubridate⁹⁰.

641 The entire measurement session was divided into different units according to the therapy
642 elements: questionnaire period before the session (Base1), contact (C), grooming (G), riding
643 (R), first trotting sequence (T1), the continued riding phase directly following the first trot
644 (T1E), second trotting sequence (T2), the continuing riding phase directly following the second
645 trot (T2E), lying (L) and farewell (F), and questionnaire period after the session (Base2). During
646 the baseline phases (Base1 and Base2), participant and horse were physically separated; HRV
647 was recorded simultaneously in both but without direct interaction, providing a reference
648 measure of non-interactive physiological co-variation.

649 *Cross-Wavelet Power Analysis*

650 Cross-Wavelet power is a statistical technique utilized in signal analysis to assess the
651 strength of the association between two time-varying signals across different frequencies and
652 time intervals^{88,91}. In contrast to wavelet coherence, which provides a normalized measure of
653 phase consistency, CWP reflects the absolute shared power between two signals across time
654 and frequency, thereby capturing the strength of physiological coupling directly. By quantifying
655 the magnitude of the signals' co-variance in the time-frequency domain, cross-wavelet power
656 enables researchers to identify regions of significant signal power and detect patterns of
657 interaction that may vary in intensity or frequency over time^{31,92,93}.

658 The HAT protocol specified maximum durations for each therapy element (C: 5 min, G:
659 5 min, R: 7 min, L: 5 min, F: 2 min). Heart rate data were divided into 30-second intervals,
660 which served as the unit of analysis nested within therapy elements. As actual element durations
661 varied slightly across participants, the number of intervals per element was not identical across
662 observations; this imbalance was accommodated by the repeated measures multilevel
663 modelling approach.

664 After data preprocessing, the wavelet analyses were conducted using the RHRV package
665 ⁸⁴ and included data interpolation, frequency analysis, and power band calculations.
666 Subsequently, the cross-wavelet power analyses were conducted with the aid of the
667 WaveletComp package ⁸⁸.

668 In contrast to standard HRV analytical approaches, cross-wavelet power analysis results
669 are derived from a continuous wavelet transform and allow for very high frequency and
670 temporal resolution, limited only by the constraints of the Heisenberg uncertainty principle ⁹⁴.
671 To strike a good balance between temporal and frequency resolution, we categorized frequency
672 into four period-based bands spanning oscillations from 2 to 24 s. In relation to the commonly
673 known high and low frequency bands derived from Fourier transform analysis, we labeled them
674 upper high frequency (UHF, 2-4s), lower high frequency (LHF, 4-8s), upper low frequency
675 (ULF, 8-18s) and lower low frequency (LLF, 16-24s). Likewise, even though temporal
676 resolution could go down to one second given that we have time series with a one-second
677 resolution, we calculated and extracted 30-second intervals of CWP data.

678 **Statistical Analysis**

679 To account for our hierarchical data structure, with 30-second intervals nested within
680 therapy segments, therapy segments nested within HAT sessions, HAT sessions nested in dyads
681 and dyads nested in groups, a specialized multilevel model approach with repeated
682 measurements (RM-MLMs) was employed. All statistical analyses were carried out as well
683 using the R statistics program ⁸² along with the nlme ⁹⁵ and the lme4 packages ^{96,97}. The choice
684 of maximum likelihood estimation, known for its robustness and unbiased estimates in large
685 samples, was chosen to ensure accuracy in the presence of nested data. For inferential statistical
686 testing of our hypotheses, various RM-MLMs were established and compared using likelihood-
687 ratio tests. Analyses of variance (ANOVAs) were conducted, with a predetermined α of 5%, to
688 determine which model better fitted the data. To account for the temporal dependency between

689 successive 30-second intervals, a first-order autoregressive correlation structure [AR(1)] was
690 included in all models.

691 This multilevel modelling approach offers several advantages over traditional repeated-
692 measures ANOVA, including the ability to handle unbalanced data structures, account for the
693 nested structure of observations within participants, and model individual differences in
694 synchronization trajectories ⁹⁸.

695 ***Assumption checks.*** We performed a series of diagnostic tests to evaluate the underlying
696 assumptions of the statistical analyses. These assessments included examining the normality of
697 residuals using QQ plots, evaluating the independence of residuals through scatterplots, and
698 assessing homoscedasticity via residual plots ⁹⁹. Furthermore, potential outliers were examined,
699 and the absence of multicollinearity was confirmed by studying correlations between predictors
700 ¹⁰⁰. These checks ensure the robustness and validity of the analyses. Given the robustness of
701 ANOVAs and RM-MLMs to deviations from normality, exceptions where a strict adherence to
702 normal distribution assumptions was not met were still considered in the statistical analysis
703 ^{101,102}.

704 **Data and code availability**

705 The data reported in this paper have been deposited at OSF and are publicly available
706 at <https://doi.org/10.17605/OSF.IO/YFWZT>.

707 All original analysis code has been deposited at OSF and is publicly available at
708 <https://doi.org/10.17605/OSF.IO/C2XGN>.

709 Any additional information required to reanalyze the data reported in this paper is
710 available from the lead contact upon request.

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970

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