Title

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Journal
Annals of Behavioral Medicine, 56(2)

ISSN
0883-6612

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Publication Date
2022-02-11

DOI
10.1093/abm/kaab037

Peer reviewed

ann. behav. med. (2022) 56:168–175
DOI: 10.1093/abm/kaab037

REGULAR ARTICLE

Social Contact Frequency and Pain among Older Adults with HIV: An Ecological Momentary Assessment Study

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Published online: 31 May 2021

Abstract

Background Social relationships are important for pain management among individuals with HIV, but the impact of daily social contact on pain responses in real-time, real-world settings has never been specifically examined.

Purpose The purpose of the present study was to examine the relationship between social contact frequency and pain, and the role of negative and positive affect in this relationship among older adults with HIV using ecological momentary assessment (EMA).

Methods A total of 66 (M_{age} = 59.3, SD = 6.3, range: 50–74) older adults with HIV completed EMA surveys that included social contact frequency, pain level, and negative and positive affect four times per day for 2 weeks. Mixed-effects regression models were used to examine concurrent and lagged associations between social contact frequency, pain, and negative and positive affect.

Results Greater recent social contact frequency was associated with less severe current pain (unstandardized $B = -0.04$, 95% CI: −0.08, −0.01, $p = .014$), while greater current pain was associated with lower subsequent social contact frequency (unstandardized $B = -0.07$, 95% CI: −0.11, −0.03, $p < .001$). Further, higher current negative affect was related to greater current pain, and this relationship was dampened by increased recent social contact frequency (unstandardized $B = -0.17$, 95% CI: −0.26, −0.08, $p < .001$). Neither negative nor positive affect was significantly associated with the relationship between current pain and subsequent social contact frequency.

Conclusions Social contact frequency and pain are bidirectionally and inversely associated among older adults with HIV. Further, recent social contact influences current pain by attenuating negative affect. Together, these results highlight the need to address social engagement in interventions for pain among older adults with HIV.

Keywords: Mobile health · Ambulatory assessment · Social engagement · Loneliness · Chronic illness · Mood

Introduction

Due to the success of antiretroviral therapy (ART), the number of older adults living with HIV has rapidly increased [1]. As a result, there is a need to better understand factors that interfere with daily functioning and quality of life in this population, particularly pain. Pain is a common and highly impairing comorbidity among people with HIV (PWH) [2]. Pain in PWH is associated with poorer physical function and ART adherence, and greater depressive symptoms, substance use, and healthcare utilization [3–7]. Pain may be especially problematic among older PWH. For example, normal aging processes contribute to pain chronification in older adults via neurodegeneration, changes in circadian rhythms, and a gradual increase in age-related inflammation, termed "inflammaging" [8]. Subsequently, increased pain can exacerbate maladaptive coping mechanisms, such as

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behavioral avoidance of daily activities and social interactions, which may further exacerbate pain in a vicious downward cycle [9]. Identifying and understanding factors that disrupt this cycle may be key to maximizing functioning among older PWH experiencing pain.

Social relationships play an important role in physical and mental health [10–12]. The measurement of social relationships is inherently complex and multifaceted. Most research focuses on social support, typically measured by perceived or received support, and social integration, which refers to one's engagement across a range of social activities [10, 13]. In regard to pain, social support and social integration tend to reduce pain experiences by attenuating negative affective experiences, which has been termed the social buffering effect [14]. It is also possible that social buffering operates through the modulation of positive affect; however, this mechanism has received less attention. There is evidence positive affect influences the experience of pain over and above that of negative affect and is associated with subsequent social engagement among individuals with chronic pain [15]. Thus, it is important to consider positive affect in addition to negative affect as a potential mechanism by which social buffering of pain may occur.

In this study, we were interested in a particular facet of social integration, social contact frequency. Social contact frequency refers to the quantity rather than the quality of interactions over a period of time [16]. There are several reasons why social contact frequency may be important among older PWH. First, older PWH are at high risk for social isolation [17], which can exacerbate pain as well as depressive symptoms, poor sleep, and suicidal ideation [18–22]. While this implies increased social contact frequency may be associated with improved outcomes among older PWH, to our knowledge this has never been specifically examined. Second, outside the HIV literature, social contact frequency mediated the relationship between physical pain and suicidal ideation among older adults [23], and is associated with decreased all-cause mortality [16]. Third, social contact frequency is a relatively objective measure. While other measures of social integration (e.g., satisfaction with one's role) and social support are important for understanding the context around social interactions, social contact frequency is less prone to subjective interpretation [16].

The purpose of the present study was to better understand the relationship between social contact frequency, pain, and affect among older adult PWH in real-time, real-world settings using ecological momentary assessment (EMA). EMA involves the repeated sampling of behavior and experiences within natural environments that allows for the examination of short-term shifts and specific contexts on outcomes within individuals. While a previous study used EMA to examine the association between social support, pain, and personality characteristics among PWH [24], this is the first study to use EMA to examine the interrelationships between social contact frequency, pain, and affect. There were two overall aims. First, we examined the impact of *recent* social contact frequency on *current* pain (Aim 1a), and the impact of *current* pain on *subsequent* social contact frequency (Aim 1b), within-persons. We expected an inverse relationship between *recent* social contact frequency and *current* pain, as well as between *current* pain and *subsequent* social contact frequency. Second, we examined interactions among social contact frequency (recent and subsequent), pain, and affect (negative and positive). That is, we examined if *recent* social contact frequency interacted with *current* affect to influence *current* pain (Aim 2a), and similarly, if *current* pain interacted with *current* affect to influence *subsequent* social contact frequency (Aim 2b). We expected that higher *current* negative affect and lower *current* positive affect would be associated with higher *current* pain, and this relationship would be weakened by increased *recent* social contact frequency. Further, we expected the relationship between higher *current* pain and less *subsequent* social contact would be strongest when *current* negative affect was high and *current* positive affect was low.

Methods

Participants

Participants were 66 PWH enrolled in the Real-Time Mobile Assessment of Daily Functioning Among Older HIV-Infected Adults study conducted at the UC San Diego HIV Neurobehavioral Research Program (HNRP). Data were collected from February 2016 to May 2019. Inclusion criteria for the parent study were: (a) being at least 50 years of age, (b) English fluency, and (c) ability to provide written, informed consent. Exclusion criteria for the parent study were: (a) serious mental illness (e.g., schizophrenia); (b) history of a non-HIV neurological disease (e.g., stroke); (c) brain injury with loss of consciousness >30 min, (d) history of severe learning disability (i.e., WRAT-4 reading score <70), or (e) positive alcohol breathalyzer or urine toxicology for drugs of abuse (other than marijuana) at the baseline visit. Additional inclusion criteria for the current study was having HIV. The UC San Diego Institutional Review Board approved all study procedures. All participants demonstrate decisional capacity [25] and provided written informed consent.

Participant demographics and clinical characteristics are presented in Table 1. Participants were 59.3 years old on average (*SD* = 6.3; range: 50–74) and mostly

Table 1. Demographic and clinical characteristics (N = 66)

	Mean (*SD*) or *N* (%)
Demographics	
Age	59.27 (6.31). Range: 50–74
Sex (women)	13 (19.7)
Years of education	13.94 (2.45)
Race/ethnicity	
Non-Hispanic White	42 (63.6%)
Black	15 (22.7%)
Hispanic	7 (10.6%)
Other	2 (3.0%)
Marital status (currently married)	7 (10.6%)
Household size (living alone)	38 (57.6%)
HIV disease characteristics	
History of AIDS (yes)	46 (69.7%)
Current CD4 count	706.00 (199.96)
Nadir CD4	188.92 (192.67)
On ART (yes)	62 (93.9%)
Undetectable plasma viral load[a]	60 (96.8%)
Estimated years living with HIV	22.76 (7.83)
Aggregate EMA ratings	
Pain	2.93 (2.12)
Negative affect	1.44 (0.67)
Positive affect	3.26 (0.92)
Number of social interactions	1.72 (0.90)

[a]Lower limit of quantification = 50 copies/ml.

non-Hispanic White (n = 42; 64%). The majority of participants were men (n = 53; 80%), which is comparable to gender demographics of HIV in the United States [26]. Most were unmarried (n = 59; 89.4%) and living alone (n = 38; 57.6%). In terms of HIV disease characteristics, participants were well controlled, with 94% (n = 62) on ART and 97% (n = 60) with undetectable HIV plasma viral loads.

Procedure and Measures

Participants completed an in-person baseline visit followed by 14 days of EMA surveys in their natural environments.

Baseline visit

At the baseline visit, participants completed neuromedical and neurobehavioral evaluations. To lessen participant burden, participants who had been enrolled in another study at the HNRP within the past 6 months did not retake the neuromedical and neurobehavioral evaluations. HIV serostatus was determined using an HIV/HCV antibody point-of-care rapid test and confirmatory western blot analyses. HIV disease characteristics were collected via structured interview (i.e., estimated duration living with HIV, historical and current ART regimen, nadir CD4 count, and historical AIDS diagnosis) and reverse transcriptase-polymerase chain reaction on blood samples (i.e., current CD4 count and plasma HIV RNA). Participants were provided a touch-screen Samsung smartphone with a 4G Android Operating System, trained on its use and on EMA survey completion, and given a smartphone Operating Manual to take home. The mobile platform used an encrypted native application framework to ensure data could not be accessed if the device was lost or stolen.

Fourteen-day EMA study period

Participants received four EMA surveys per day on the study smartphone. Completion time for each survey was about 3 min. The delivery of EMA surveys occurred at random intervals separated by approximately 3 h and was timed to accommodate each participant's sleep-wake schedule. At each survey delivery time, participants were alerted every 2 min until they responded or until the survey deactivated (i.e., 16 min after the initial alert). Every EMA survey included inquiries about social contact frequency, pain, and affect. To quantify social contact frequency, participants were asked, "Since the last alarm, how many times did you socialize with someone else [e.g., spent more than five minutes talking/communicating with someone else]?" with five response options from "0 (you had no interactions)" to "4 or more interactions"). The duration or modality of contact was not captured. Note that this question refers to social interactions experienced between the previous and current prompt. Pain was assessed by the question, "What is your pain level right now?" for which participants responded using a visual analog scale from 1 (minimal or no pain) to 10 (severe pain). Affect was assessed via five individual survey items asking participants to rate their current feelings of happiness, depression, worthlessness, anxiety, and worry (e.g., "I feel happy…") on a Likert-type scale from 0 (not at all) to 4 (very much). Ratings from depression, worthlessness, anxiety, and worry were averaged to create a summary score reflecting negative affect. Ratings of happiness were used as a proxy for positive affect.

Statistical Analyses

To determine potential demographic covariates for all analyses examining the primary study aims, Pearson r correlations or t-tests were used to explore the relationships between continuous and dichotomous demographic characteristics (i.e., age, education level, sex, and race/ethnicity [non-Hispanic White vs. other], marital status [currently married vs. unmarried], household size [living alone vs. living with one or more persons]) and average levels of EMA-assessed variables of interest. To examine the primary aims (i.e., relationships between social contact frequency and pain), we conducted both concurrent and lagged analyses. In concurrent analyses with current pain as the outcome, social contact frequency represents *recent* social contact frequency because the prompt specified the number of social interactions since the last survey. In lagged analyses, we examined current pain as a predictor of *subsequent* social contact frequency (i.e., number of social interactions reported at the next survey).

For the first study aim, bivariate linear mixed-effects models were conducted to examine the unadjusted within-person relationships between recent social contact frequency and current pain (Aim 1a) and between current pain and subsequent social contact frequency (Aim 1b) controlling for between-person levels of pain and social contact frequency. To examine Aim 2a, linear mixed-effects models were used to determine the within-person independent and interactive effects of recent social contact frequency and current affect (negative and positive) on current pain level, covarying for time of day (i.e., 1 = morning, 2 = midday, 3 = afternoon/evening, 4 = night time), average affect, and average social contact frequency [27]. Current positive and negative affect were person-mean centered. These models included person-specific random intercepts and random slopes for social interactions and affect. To examine Aim 2b, linear mixed-effects models were used to determine the within-person independent and interactive effects of current pain and current affect (negative and positive) on subsequent social contact frequency, covarying for time of day, average pain level, and average affect. Current pain and affect were person-mean centered. For these lagged analyses, pain and affect responses from the last survey of each day were not used to predict social contact frequency from the next morning survey. These models included person-specific random intercepts and random slopes for pain and affect. Both unstandardized and standardized regression estimates are reported. Standardized regression estimates were used as an estimate of effect size for individual predictors [28]. All analyses were conducted using R, version 3.5.0. Multilevel models were examined using the "lme4" package [29].

Results

Being currently married and living with one or more persons was associated with greater social contact frequency (p's > .05). Other demographic variables (age, sex, years of education, race/ethnicity) were unrelated to social contact frequency, pain, negative affect, or positive affect (p's < .05). Bivariate within-person relationships among recent and subsequent social contact frequency, current pain, and current negative and positive affect are shown in Table 2. In regard to Aims 1a and 1b, higher recent social contact frequency was associated with lower current pain (unstandardized $B = -0.04$, 95% CI: −0.08, −0.01, $p = .014$; standardized $B = -0.06$), and higher current pain was associated with less subsequent social contact frequency (unstandardized $B = -0.07$, 95% CI: −0.11, −0.03, $p < .001$; standardized $B = -0.03$), respectively, controlling for between-person levels of pain and social contact frequency. To verify the strength of these associations, two sets of sensitivity analyses were performed by including average negative and positive affect as covariates, and by including marital status and household size as covariates to these models. Findings were unchanged.

Results of all four linear-mixed effects models associated with Aim 2 are displayed in Table 3. In regard to Aim 2a, the linear mixed-effects model examining the within-person independent and interactive effects of recent social contact frequency and current negative affect on current pain revealed a significant interaction (unstandardized $B = -0.17$, 95% CI: −0.26, −0.08, p

Table 2. Results of bivariate linear mixed-effects models examining within-person relationships among EMA-assessed variables of interest.

Predictors	Subsequent social contact frequency[a]	Pain level	Negative affect	Positive affect
Recent social contact frequency	0.18 (0.14, 0.22)**	−0.04 (−0.08, −0.01)*	−0.01 (−0.02, 0.00)	0.10 (0.08, 0.01)**
Pain level (person-centered)	−0.07 (−0.11, −0.03)**	–	0.04 (0.03, 0.05)**	−0.09 (−0.11, −0.07)**
Negative affect (person-centered)	−0.10 (−0.24, 0.04)	0.43 (0.31, 0.55)**	–	−0.54 (−0.61, −0.46)**
Positive affect (person-centered)	0.14 (0.07, 0.02)**	−0.21 (−0.26, −0.16)**	−0.11 (−0.13, −0.09)**	–

Note. Values are unstandardized regression coefficients (95% CI).

[a]Values in this column represent the lagged relationship between each variable and subsequent social contact frequency.

*$p < .05$; **$p < .001$.

Table 3. Results of linear mixed effects models examining relationships among social contact frequency, pain, and negative and positive affect.

	Unstandardized estimate (95% CI)	Standardized estimate (95% CI)	*p*-value
Outcome: pain level (Aim 2a)			
Time of day	0.037 (0.003, 0.070)	0.017 (0.002, 0.033)	.032
Average recent social contact frequency	0.284 (−0.278, 0.845)	0.107 (−0.105, 0.319)	.326
Average negative affect	1.143 (0.391, 1.894)	0.307 (0.105, 0.509)	.004
Recent social contact frequency	−0.029 (−0.072, 0.013)	−0.018 (−0.044, 0.008)	.181
Negative affect (person-centered)	0.629 (0.361, 0.898)	0.087 (0.050, 0.124)	<.001
Recent social contact × negative affect	−0.169 (−0.260, −0.077)	−0.051 (−0.079, −0.023)	<.001
Outcome: pain level (Aim 2a)			
Time of day	0.032 (−0.001, 0.065)	0.015 (−0.001, 0.031)	.057
Average recent social contact frequency	0.122 (−0.486, 0.730)	0.046 (−0.183, 0.275)	.696
Average positive affect	−0.234 (−0.829, 0.361)	−0.089 (−0.315, 0.137)	.443
Recent social contact frequency	−0.001 (−0.046, 0.046)	−0.001 (−0.028, 0.027)	.987
Positive affect (person-centered)	−0.245 (−0.385, −0.105)	−0.074 (−0.116, −0.032)	.001
Recent social contact × positive affect	0.021 (−0.022, 0.064)	0.014 (−0.016, 0.044)	.349
Outcome: subsequent social contact frequency (Aim 2b)			
Time of day	−0.127 (−0.181, −0.073)	−0.073 (−0.104, −0.042)	<0.001
Average pain level	0.088 (−0.017, 0.194)	0.129 (−0.025, 0.283)	0.106
Average negative affect	−0.421 (−0.757, −0.084)	−0.185 (−0.334, −0.037)	0.017
Pain level (person-centered)	−0.057 (−0.116, 0.001)	−0.046 (−0.092, 0.001)	0.064
Negative affect (person-centered)	−0.026 (−0.211, 0.158)	−0.006 (−0.048, 0.036)	0.781
Pain level × negative affect	−0.033 (−0.137, 0.072)	−0.011 (−0.047, 0.024)	0.540
Outcome: subsequent social contact frequency (Aim 2b)			
Time of day	−0.126 (−0.180, −0.072)	−0.072 (−0.103, −0.041)	<0.001
Average pain level	0.052 (−0.047, 0.152)	0.077 (−0.069, 0.222)	0.307
Average positive affect	0.390 (0.159, 0.621)	0.244 (0.100, 0.388)	0.002
Pain level (person-centered)	−0.056 (−0.111, −0.001)	−0.045 (−0.088, 0.000)	0.052
Positive affect (person-centered)	0.132 (0.050, 0.214)	0.066 (0.025, 0.106)	0.003
Pain level × positive affect	0.045 (−0.005, 0.095)	0.031 (−0.003, 0.064)	0.077

< .001; standardized $B = -0.051$). This finding was unchanged by including marital status and household size as covariates. When specifying recent social contact frequency as the moderator, simple slopes analysis revealed that the within-person relationship between current negative affect and current pain was weakened at times when recent social contact frequency was high (Fig. 1). When examining positive affect, results showed that there was no significant interaction between subsequent social contact frequency and current positive affect on current pain within persons ($p = .35$). In regard to Aim 2b, the interaction between current pain and current negative affect did not significantly predict subsequent social contact frequency within persons ($p = .54$). The interaction between current pain and current positive affect, however, was marginally significant within persons ($p = .077$; Table 3). An exploratory simple slopes analysis showed that the negative within-person relationship between current pain and subsequent social contact frequency was weakest at times when current positive affect was high.

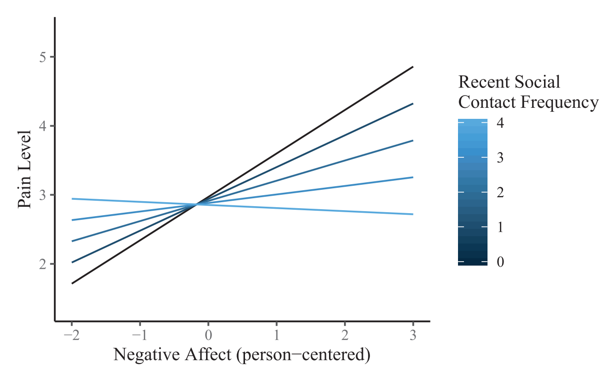

Fig. 1. The within-person relationship between current negative affect and current pain depends on recent social contact frequency.

Discussion

This study sought to better understand the association between social contact frequency, pain, and affect among older adult PHW in real-time, real-world settings using advances in mobile technology. Recent social contact frequency was inversely associated with current pain, and current pain was inversely associated with subsequent social contact frequency. Further, there was a significant within-person effect that demonstrated higher current negative affect was related to higher current pain, and this relationship was weakest at times when recent social contact frequency was high. The influence of pain on subsequent social contact frequency was not significantly moderated by within-person variability in current positive or negative affect, suggesting current pain relates to less subsequent social interactions regardless of affect.

While previous work has shown an association between social contact frequency and pain [23], to our knowledge this is the first study to report the bidirectional impact of social contact frequency and pain within-persons using EMA. Given that we covaried for average levels of pain, affect, and social contact frequency, as well as marital status and household size, our findings suggest that significant within-person effects were independent of between-person variability in these variables or living situation. That is, our findings demonstrate how observable changes within a person relate to behavioral patterns at the individual level. This highlights the importance of considering the role of pain in the context of social isolation among older PWH. Specifically, pain may exacerbate social isolation in a downward spiral, such that greater pain may lead to less social contact, which in turn leads to more pain. Given the importance of maintaining social networks for promoting QoL and successful aging in PWH [30], it is especially important that clinicians assess pain severity and interference when discussing social activity in older PWH.

The relationship between negative affect and pain is well-established, with many studies showing negative affective states are generally associated with greater pain sensitivity (see [31] for a review). Results showed that the relationship between current negative affect and current pain was weakened by recent social contact frequency (i.e., social buffering). Again, we would like to emphasize that this effect was found within-persons. This is in contrast to a previous EMA study that showed the association between increased social support and lower pain varied by personality characteristics (attachment-related insecurity) among PWH [24], a between-person factor. Our findings suggest that individual variations in negative affect influence when individuals benefit from social contact. For example, older PWH may be more likely to experience social buffering on pain at times when negative affect is high. Or said the opposite, when social contact has been less recent, a strong and positive association between current pain and current negative affect would be expected.

Although the moderating role of current affect in the association between higher current pain and less subsequent social contact frequency was not statistically significant, there was a non-significant trend whereby the negative impact of current pain on subsequent social interactions was diminished at times when current positive affect was high. Negative and positive affect are related but distinct constructs [32], and a 2015 review highlights the importance of considering the protective role of positive affect in the management of pain [15]. Nonetheless, our findings suggest additional processes influence the relationship between current pain and subsequent social interactions. One possibility is pain expectancy. In both cross-sectional and longitudinal studies, pain expectancy was associated with greater behavioral avoidance [33, 34], even after controlling for pain level and negative affect [35]. Indeed, among individuals with chronic pain, the threat of increased future pain prevented social engagement with family and friends [36]. This was also found among PWH, who reported avoidance of social activity as a self-management strategy for pain [37]. Thus, an important clinical implication from the current study is that pain management treatments for PWH may benefit from including psychoeducation on the benefits of social activity as well as interventions to increase social interactions.

It is important to point out that not all social interactions confer pain benefits but may instead exacerbate pain. Among research on couples, negative interactions (e.g., expressing frustration and anger about pain) and solicitous responses (e.g., encouragement to be less active) from significant others are generally associated with greater pain levels and disability among individuals with chronic pain [38, 39]. Interaction types associated with decreased pain behavior and improved functioning include validating responses and emotional disclosure [40, 41]. Although we cannot directly speak to the content, quality, or duration of interactions in this study, our bivariate analyses revealed a significant positive association between recent social contact frequency and current positive affect, suggesting that on average interactions were likely positive. This is fitting with past research showing social interactions tend to be more supportive among older adult PWH relative to younger adult PWH [42]. Nonetheless, because social interactions may have a buffering or amplifying effect on pain, it is important that clinical interventions carefully consider the dynamics within social networks and devise approaches to minimize negative consequences (e.g., decreasing the impact of negative interactions, managing solicitous responses) and maximize positive consequences (e.g., encouragement for emotional disclosure where appropriate, strategies for increasing the enjoyment and meaning of interactions).

In general, our findings suggest being less socially engaged is associated with greater pain in older PWH. While data were collected prior to the COVID-19 pandemic, this is especially relevant in the context of the ongoing pandemic where social isolation and loneliness are likely to be exacerbated. This is compounded in older PWH given they are at high risk for adverse events if infected by the virus and may be asked or required to adhere to stay-at-home orders for the foreseeable future. It may be imperative to implement social outreach programs in this vulnerable group to help manage pain, as well as to help preserve overall physical and mental health functioning.

There are several strengths to the current study. First, the study adds to the limited literature on the social buffering effect on pain among older PWH and provides additional rationale for addressing social contact frequency in clinical intervention approaches. Second, the social buffering effect on pain was moderated by a within-person variation of negative affect, suggesting high generalizability. Third, the present study provides evidence for the bidirectional influence of pain and social interactions in older PWH. The study also has limitations. First, due to our modest sample size, additional studies with larger samples sizes are needed to replicate findings. Second, chronic pain was not an inclusion criterion for the study. Our findings may not completely generalize to PWH who have chronic pain. Third, we did not have access to other variables that are important to consider when examining social relationships. In addition to adding measures of perceived and received social support, we also encourage future research to collect information on the form of interactions (in-person vs. digital), which is especially relevant given COVID-19 related physical distancing requirements. Finally, only one variable, happiness, was used as a proxy for positive affect. Other components of positive affect include joy, contentment, and excitement [43]. Future studies are encouraged to capture additional components of positive affect to more thoroughly understand its contribution to the association between social engagement and pain.

Conclusions

This study demonstrated the within-person association between current negative affect and current pain was buffered by recent social contact frequency. Higher current pain was also associated with fewer subsequent social interactions; however, this was not dependent on current levels of negative or positive affect. Methods to increase social contact frequency should be considered in pain management interventions for PWH. To accomplish this, additional research is encouraged to identify barriers and facilitators of social engagement despite the presence of pain.

Funding

Funding for this project came from the National Institute of Mental Health K23 MH107260 (PI: Moore) as well as P30MH062512. Dr Herbert is supported by VA RR&D CDA Grant 1IK2RX002807-01A2 and partially supported by R01DK106415 from National Institute of Diabetes and Kidney Disease. Dr Wooldridge is supported by the VA Office of Academic Affiliates advanced fellowship in women's health. Ms. Paolillo is supported by T32 AA013525 and F31AA027198. Dr Moore is supported by R01AG062387 and R21MH116104. The views expressed in this paper are those of the authors and do not reflect the official policy or position of the funding agency, Department of Veterans Affairs, the United States Government, or any institutions with which the authors are affiliated.

Compliance with Ethical Standards

Authors' Statement of Conflict of Interest and Adherence to Ethical Standards Dr Moore is a co-founder of KeyWise AI and a consultant for NeuroUX. All other authors declare no conflicts of interest.

Authors' Contributions M.S.H. conceived the study, interpreted the data, and drafted the manuscript. J.S.W. assisted in the conceptualization of the study, interpreted the data, and contributed to manuscript drafting. E.W.P. analyzed the data and contributed to manuscript drafting. C.A.D. and R.C.M. assisted in the design of the study and critically revised the article for important intellectual content. All authors approved the final manuscript.

Ethical Approval All procedures were conducted in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinski Declaration of 1975, as revised in 2000.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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