DR. ALEXIES DAGNINO-SUBIABRE (Orcid ID : 0000-0002-9909-9826)

Article type : Special Issue Article

C-Low Threshold Mechanoafferent Targeted Dynamic Touch Modulates Stress Resilience in Rats Exposed to Chronic Mild Stress

Susannah C. Walker[1], Antonia Cavieres[2], Valentín Peñaloza-Sancho[2], Wael El-Deredy[3], Francis P. McGlone[1,4], Alexies Dagnino-Subiabre[2]*

[1]Research Centre for Brain & Behaviour, Liverpool John Moores University, Liverpool, UK

[2]Laboratory of Stress Neurobiology, Centre for Integrative Neurobiology and Pathophysiology, Institute of Physiology, Faculty of Sciences, Universidad de Valparaíso, Valparaíso, Chile.

[3]Center for Research and Development in Health Engineering, Universidad de Valparaíso, Valparaíso, Chile.

[4]Institute of Psychology, Health & Society, University of Liverpool, Liverpool, UK

***Corresponding author:**

Alexies Dagnino-Subiabre, Ph.D.

Laboratory of Stress Neurobiology

Faculty of Sciences

Universidad de Valparaíso

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/EJN.14951

Gran Bretaña 1111, Playa Ancha
Valparaíso, Chile
Tel.: +56-032-2508020
FAX: +56-032-2281949
E-mail: alexies.dagnino@uv.cl

Number of text pages: 35

Number of scheme: 1

Number of figures: 5

Running Title: Gentle stroking stimulation promotes stress resilience.

ABSTRACT

Affiliative tactile interactions buffer social mammals against neurobiological and behavioral effects of stress. The aim of the present study was to investigate the cutaneous mechanisms underlying such beneficial consequences of touch by determining whether daily stroking, specifically targeted to activate a velocity/force tuned class of low-threshold c-fiber mechanoreceptor (CLTM), confers resilience against established markers of chronic unpredictable mild stress (CMS). Adult male *Sprague Dawley* rats were exposed to two weeks of CMS. Throughout the CMS protocol, some rats were stroked daily, either at CLTM optimal velocity (5cm/s) or outside the CLTM optimal range (30cm/s). A third CMS exposed group did not receive any tactile stimulation. The effect of CMS on serum corticosterone levels, anxiety- and depressive-like behaviors in these three groups was assessed in comparison to a control group of non-CMS exposed rats. While stroking did not mitigate the effects of CMS on body weight gain, CLTM optimal velocity stroking did significantly reduce CMS induced elevations in corticosterone following an acute forced-swim. Rats receiving CLTM optimal stroking also showed significantly fewer anxiety-like behaviors (elevated plus-maze) than the other CMS exposed rats. In terms of depressive-like behavior, while the same velocity specific resilience was observed in a forced-swim test (FST) and social interaction test both groups of stroked rats spent significantly less time interacting than control rats, though they also spent significantly less time in the corner than non-stroked CMS rats. Together, these findings support the theory CLTMs play a functional role in regulating the physiological condition of the body.

Keywords: stress, affective touch, resilience, C-tactile afferent, Low threshold mechanoreceptor.

INTRODUCTION

An acute stress response initiates a cascade of physiological and behavioural changes, mediated by the hypothalamic pituitary adrenal axis (HPA) and sympathetic nervous system (SNS), which allow an individual to respond adaptively to environmental challenge (McEwen, 1998; Franklin et al., 2012). In highly social species, affiliative touch has been reported to modulate this reaction (Vannorsdall et al., 2004; Parker et al., 2006; Ditzen et al., 2007; Walker, 2010; Morrison, 2016).

Allostatic load describes physical and mental wear-and-tear due to repeated HPA and SNS activation resulting from exposure to repeated or chronic stress, leading to changes in brain function and behavior which increase the risk of illnesses such as depression and anxiety disorders (McEwen & Akil, 2020). The resilience conferring effects of affiliative touch have been confirmed in rodent studies where licking and grooming of rat pups by their mothers modulates how the rat, as an adult, responds to stressful events (Caldji *et al.*, 1998; Meaney, 2001; Champagne *et al.*, 2003; Champagne & Meaney, 2007; Champagne, 2008; Hellstrom *et al.*, 2012). In the absence of this maternal input the effects can be effectively mimicked by stroking the animal with a soft brush (Van Oers *et al.*, 1998; Gonzalez *et al.*, 2001). While the cutaneous mechanisms underlying the beneficial consequences of affiliative touch remain to be addressed, a range of previous studies have reported that low intensity stimulation of somatosensory nerves, through stroking touch, warmth and light pressure, modulates HPA and SNS activity, decreasing blood pressure and cortisol levels and stimulating oxytocin and endogenous opioid release (Araki et al., 1984; Stock and Uvnas-Moberg, 1988; Uvnäs-Moberg et al., 1996, 2014; Lund et al., 1999; Walker and McGlone, 2013; Nummenmaa et al., 2016).

In recent years, the electrophysiological study of human skin nerves has led to the identification and characterization of a class of low-threshold C-fiber mechanoreceptors (CLTMs) (Nordin, 1990; Vallbo *et al.*, 1999), that respond optimally to low force, dynamic touch. These CLTMs are temperature and velocity tuned and their preferred stimulus is skin temperature stroking around ~5 cm/s (Löken *et al.*, 2009;; Ackerley *et al.*, 2014). Neuroimaging studies have shown that gentle stroking touch applied to hairy skin, where CLTMs are abundant, but not palmar skin, where in humans CLTMs have not been found, reliably produces neural activation in affective and reward related brain regions (Olausson *et al.*, 2002; Mcglone *et al.*, 2012; Gordon *et al.*, 2013). Their

response characteristics and central projections make CLTMs ideally suited to form the first stage of encoding socially relevant and rewarding tactile information resulting from affiliative behaviors. The affective touch hypothesis proposes CLTMs have an evolutionarily conserved function in the formation and maintenance of social bonds (Morrison *et al.*, 2010; Olausson *et al.*, 2010; McGlone *et al.*, 2014).

Evidence for the specific rewarding value of CLTM activation comes from a study showing pharmacogenetic activation of unmyelinated sensory nerves, which respond preferentially to massage like stroking, but not noxious mechanical stimulation, promoted the formation of conditioned place preference, indicating their activation carries a positively reinforcing value (Vrontou *et al.*, 2013). Furthermore, Maruyama *et al* (2012) reported stroking applied at a CLTM optimal velocity of approximately 5 cm/s to the back, limbs, or abdomen evoked dopamine release in the nucleus accumbens of both awake and anesthetized rats. In contrast, a noxious pinching stimulus had no such effect.

CLTMS, have been identified in the hairy skin of all mammals so far studied (Zotterman, 1939; Douglas & Ritchie, 1957; Bessou *et al.*, 1971; Iggo & Kornhuber, 1977; Kumazawa & Perl, 1977; Lynn & Carpenter, 1982) and evidence to date indicates their sensory information is transmitted to the brain via projection neurons located in lamina I of the spinal cord (Lu & Perl, 2005; Andrew, 2010; though see Abraira *et al.*, 2017). Their functional anatomy signifies CLTMs belong to a set of small diameter primary sensory nerves which together form an ascending pathway which represents the physiological condition of the body and thus contributes to homeostasis (Craig, 2003; Björnsdotter et al., 2010; Strigo and Craig, 2016). In the rat, most ascending lamina I activity is relayed by the spinoparabrachial pathway which projects to the medulla, including the nucleus of the solitary tract (NST), the hypothalamus, amygdala, and bed-nucleus of the stria terminalis (BNST) (Bernard *et al.*, 1993; Alden *et al.*, 1994; Bester *et al.*, 1997; Polgár *et al.*, 2010; Wercberger & Basbaum, 2019). In addition, somatosensory information from this pathway is transmitted to the insula via both the ventroposterior medial and the posterior triangular thalamic nuclei (Gauriau & Bernard, 2004; Al-Khater & Todd, 2009). Thus, through these subcortical projections, this cutaneous afferent input is well placed to modulate affective, autonomic & endocrine functions (Strigo & Craig, 2016), providing

a plausible neural mechanism by which affiliative, tactile interactions can buffer physiological responses to stress (Morrison, 2016).

CMS protocols have been developed as validated rodent models for inducing anhedonia and disruptions of HPA function (Moreau, 1997; Cerqueira *et al.*, 2007; Castro *et al.*, 2012; Ortiz & Conrad, 2018). Experimentally, tactile stimulation, in the form of handling, has previously been reported to decrease behavioral and endocrine markers of chronically stressed rats (Aulich *et al.*, 1974; Costa *et al.*, 2020). The aim of the present study was to test the hypothesis that CLTMs play a functional role in the physiological regulation of the body's responses to stressors and that stroking at CLTM optimal velocity (5 cm/s), but not faster non CLTM optimal (30 cm/s) strokes, will buffer rat's neuroendocrine and behavioral responses to CMS.

MATERIALS AND METHODS

Ethics Statement

All procedures, animal maintenance and experimentation were approved by the Institutional Animal Ethics Committee of the Universidad de Valparaíso (Anillo de Ciencia y Tecnología Grant Nº ATC 1403) and were in strict agreement with animal care standards outlined in National Institutes of Health (USA) guidelines. Efforts were made to minimize the number of rats used and their suffering.

Animals

Male *Sprague Dawley* rats (340-350 g, 70 days old at the start of the experiment), commercially acquired (Charles River Laboratories, Wilmington, USA) were used as subjects in this experiment. All rats were maintained under a 12-h light–dark cycle (lights on at 8:00 am) and provided with water and food (Prolab RMH 3000, LabDiet®, MO, USA) ad libitum. Experiments were performed during the light phase. Animals were maintained in a temperature and humidity-controlled room (22 ± 1°C, 55%), and housed in groups of three. On a daily basis, each rat was removed at 10.00 h from their home cage by hand and transferred to another cage on a digital scale to be weighed. The experimenters who conducted the handling procedure were different to those who applied the stress protocol. This procedure was applied to all rats from weaning until the end of the experiment.

Animals under the CMS protocol were separated from non-stressed animals and kept in a different room. Body weights were measured daily throughout the stress protocol.

Experimental Design

Scheme 1 shows the timeline of the experimental design. In Experiment 1, we evaluated the effects of stroking and CMS on plasma corticosterone levels and body weight gain. In Experiment 2, locomotor activity (open field), anxiety (elevated plus maze), depressive-like behaviors (forced swim test), and social interaction were determined in non-stressed rats and animals that were exposed to CMS.

Tactile Stimulation

Tactile stimulation was always applied by the same experimenter who performed the daily handling to weigh rats. Rats received 10 minutes of dorsal stroking, from head to tail, immediately prior to the application of the daily CMS stressor. Stroking was applied through the experimenter's hands at one of two velocities, CLTM optimal 5 cm/s or Non-CLTM optimal 30 cm/s. The velocity of the experimenter's hand movement during stroking stimulation was quantified using the EthoVision XT video software. A webcam connected to a computer was installed in front of the experimenter for the experimenter to be able to see the velocity of their hand movement in the Ethovision software during the stroking stimulation.

Rats in the non-stress and non-stroking groups did not receive any stroking, they were only subjected to the daily handling procedure.

Chronic Mild Stress

The stress protocol used in this study was modified from previous studies (Castro *et al.*, 2012; Jacinto *et al.*, 2016). Rats from the stress group were exposed each day to one of seven stressors in an unpredictable order for 14 consecutive days. The protocol included a psychogenic stressor, exposure to cat odor (2,5-dihydro-2,4,5-trimethylthiazoline) for 1 h, and six physical stressors, acoustic stimulation (noise bursts: 78-115 dB, 20-40 ms, intertrial intervals from 4 to 22 s, 13 s average) for 15 min, shaking (cage movement) for 1 h, cold air stream (18 ºC) for 1 h, restraint stress for 1 h, inverse light and dark cycle, over a 48 h period, exposure to overcrowding under a bright light (six rats in a standard home cage, 1000 lux) for 2 h. After each stress session rats were returned to their home cage. Body weight was measured at the same time of day as a stress marker.

Experiment Nº 1

Corticosterone levels

This experiment was designed to evaluate the effects of the CMS protocol and stroking stimulation on serum corticosterone. Six rats were used in each experimental group (Non-stress, n = 6; Stress, n = 6; Stress + 5 cm/s, n = 6; Stress + 30 cm/s, n = 6).

Serum corticosterone levels were measured before and after the stimulation of the HPA axis by a new acute stressor (forced swim). Extraction of the blood samples were made between 10.00 am

at 1:00 pm. Each rat was picked up from its home cage and gently held in the hand of the experimenter for extraction of the blood samples from the tail vein. Immediately after, the rats were exposed to 60 seconds of forced swim, in a plastic beaker (46 cm deep, 25 cm in diameter) containing 30 cm of water (20 ± 1 ºC). The rats were then moved to a heated holding cage for 10 min, after which two new blood samples were obtained 15 and 90 minutes after initial extraction of the blood sample. Blood samples (50 µL) were collected in heparinized tubes and centrifuged (Model # MiniSpin Plus; Eppendorf AG, Hamburg, Germany) to obtain serum. Corticosterone was measured by an Enzyme Immunoassy kit (Corticosterone Competitive ELISA Kit, Catalog #EIACORT, ThermoFisher Scientific Inc, Loughborough, UK). Optical density values were determined at 450 nm using a micro-plate reader (Tecan GENios™, Tecan Group Ltd., Switzerland).

Experiment Nº 2

In this experiment, the effects of the CMS protocol and stroking on body weight, anxiety levels and depressive-like behaviors were evaluated. A new set of animals was used for this experiment (Non-stress, n = 9; Stress, n = 9; Stress + 5 cm/s, n = 9; Stress + 30 cm/s, n = 9). We determined the difference between body weight at the beginning post-natal day (PND) 70 and end of the experiments (PND 84) (Scheme 1).

Behavioral Testing

Prior to the experiments, rats were habituated to the testing room for 30 minutes on 3 consecutive days. Habituation and behavioral examination were carried out in a soundproof and temperature-controlled (21 ± 1 ºC) room. Rats were naive to the all behavioral tests.

The behavior of each rat was recorded with a webcam (WideCam 1050, Genius, Taiwan, China) and videos were automatically analyzed using EthoVision® XT version 15 (Noldus, Wageningen, The Netherlands). All mazes were cleaned with a 5% ethanol solution after each trial.

Open Field Test

Locomotor activity was evaluated using the open field test. Each animal was placed in the center of a black Plexiglass cage (70 x 70 x 40 cm) for 5 minutes. The background noise level in the open

field was 40 dB SPL (Precision sound level meter, Model#1100, Quest Technologies, Oconomowoc, WI, USA) and the arena was illuminated to 200 lux (measured by a digital lux meter, Model # LX-1010B, Weafo Instrument Co., Shanghai, China).

Average speed and total distance travelled were analyzed from video. The arena was divided into sixteen equal squares. The central zone was defined within the four central squares and the rest of the squares correspond to the border zone. Time spent in the center and border zone of the arena were analyzed from video. Entry to a zone was defined as occurring when the rat placed all four limbs onto the center and periphery.

Elevated-Plus Maze Test

Anxiety-like behavior was tested using an elevated plus-maze paradigm. Each rat was placed individually in an elevated plus-maze, consisting of two closed arms (60 x 15 x 20 cm each), two open arms (60 x 15 cm each), and a central platform (15 x 15 cm), arranged so that the two arms of each type were opposite to each other. The maze was elevated 100 cm above the floor. The lighting was 210 lux in the closed arms and 300 lux in the open arms. At the beginning of the 5minute test, rats were placed at the center of the maze, facing an open arm. Entry into an arm was defined as having occurred when the rat placed all four limbs onto the arm floor. Time spent in the open arm of the maze and the ratio of open to total arm entries (open/total×100) were used as measures of anxiety-like behaviors.

Forced Swim Test

Low mood or dysthymia, a core symptom of major depression, was evaluated in rats through FST (Wang et al., 2017). Rats were individually immersed for 5 minutes on a see-through Plexiglas cylinder (25 cm in diameter, 46 cm height), filled with 30 cm of water at 25°C. Behavior was recorded and later manually scored using EthoVision® XT. Three types of behavior were assessed: floating, climbing, swimming. Floating behavior was defined as minimal movements needed for the rat to keep its head above water and maintaining a vertical position of at least 10° from the surface.

Social Preference-Avoidance Test

Given the social focus of the present study, social interaction was used as an ecologically relevant reinforcer to evaluate for anhedonia or inability to feel pleasure (Iturra-Mena et al., 2019). A social interaction paradigm was used to test social behavior of the rats (Francis *et al.*, 2015; Zoicas & Neumann, 2016). The animals were placed in an open field with the same characteristics described in the open field test section, which contained a transparent perforated chamber (25 x 15 cm) in a designated interaction zone, which was located in the middle on one side of the open field (non-social target). The interaction zone encompasses rectangular area projecting 2.5 cm around of the perforated chamber. The corner zones cover a 20 cm x 20 cm area projecting from both corners joints opposing the perforated chamber. In the habituation phase, the rats were free to explore for 5 minutes and time spent in the interaction zone was measured. Immediately afterwards, a novel rat (male *Sprague Dawley* of similar age and weight, social target) was placed inside the perforated chamber, located in the interaction zone. In the social interaction phase, the experimental rat was then allowed to explore the maze for 15 minutes. Time spent in the interaction and corner zones, and the percentage of social interaction [(100 x time of interaction with social target present)/900] were determined. The experimenter was blind to group conditions of the rats.

Statistical analyses

All variables met the criteria for normal distribution (Shapiro-Wilk test) and homoscedasticity (Levene test) and were thus analyzed with parametric statistics.

Body weight gain, locomotor activity, anxiety, depressive-like behaviors, and social interaction were analyzed with T-Students to compare non-stress and stress group, and one-way ANOVA to compare between the stress groups (stress, stress+ 5 cm/s, and stress + 30 cm/s). The dependent variables were body weight gain from PND 70 to PND 84, distance travelled and average speed (locomotor activity), time spent in the center and border zone of the open field (anxiety-like behaviors), time in the open arm and open arm entry ratio (anxiety-like behaviors), time floating and climbing in the FST (depressive-like behaviors) and time spent socially interacting.

Results of plasma corticosterone levels were analyzed with repeated measures two-way ANOVA. The factors were stroking velocity/CMS and the time points, before vs. after acute swim stress. Serum corticosterone levels was the dependent variable. Bonferroni post hoc test for multiple comparisons was used to analyze all results since the criteria of normality and homoscedasticity were met in all variables.

Statistical analyses were performed using Prism 8 (GraphPad Software Inc., La Jolla, CA, USA) and IBM SPSS® (IBM Corp, New York, NY, USA). A probability level of 0.05 or less was accepted as significant. Results were expressed as mean ± mean standard error (SEM).

RESULTS

Effects of CMS on stress markers.

Figure 1 shows the effects of CMS protocol and stroking velocity on body weight gain and serum corticosterone levels. Rats exposed to CMS gained less weight between PND 70 to PND 84 than non-stressed rats ($p < 0.01$). Stroking velocity did not affect body weight gain in animals of stressed groups ($p = 0.82$).

For serum corticosterone analysis, the repeated measures two-way ANOVA analysis showed a significant time x group interaction ($F_{(6,30)}$=6.36, p<0.001). We found a main effect of the time point ($F_{(2,10)}$=183.3, p<0.001). Subsequent post hoc analysis showed that 15 minutes after acute swim stress, serum corticosterone levels increased in stressed rats compared to non-stressed animals ($p < 0.001$). Interestingly, rats exposed to CMS protocol and stroked at 5 cm/s had serum corticosterone levels comparable with non-stressed rats ($p > 0.9999$), while stressed rats stroked at 30 cm/s had significantly higher corticosterone levels than non-stressed rats ($p < 0.001$). Ninety minutes after stimulating the HPA axis with forced swimming, all the rats in the experimental groups returned to the basal levels of serum corticosterone ($p > 0.9999$).

Locomotor activity and anxiety-like behaviors.

CMS protocol did not affect locomotor activity, as measured by the distance travelled ($p = 0.94$) and average speed ($p = 0.78$) that rats explored the open field (Figure 2A,B).

Chronically stressed rats spent significantly less time in the center zone ($p < 0.05$) and more time in the border zone ($p < 0.05$) than did non-stressed rats (Figure 2C,D). This effect of stress was prevented when the stressed rats were stroked at speed of 5 cm/s (time in the center zone, $p < 0.05$; time in the border zone, $p < 0.05$) (Figure 2C,D).

Figure 3 shows a significant effect of CMS and stroking stimulation on time spent in the open arm and the ratio of open to total arm entries in the elevated plus maze test. Stressed rats spent significantly less time on the open arm than non-stressed rats ($p < 0.001$). This effect of stress was prevented when the rats were stroked at speed of 5 cm/s ($p < 0.001$) (Figure 3A).

Rats exposed to CMS had significantly lower ratio of open to total arm entries than non-stressed rats ($p < 0.001$) (Figure 3B). This effect of CMS was prevented when stressed rats were stroked at 5 cm/s ($p < 0.01$), but 30 cm/s strokes did not have the same effect ($p = 0.059$) (Figure 3B).

Depressive-like behaviors.

CMS significantly increased the floating time compared to non-stressed rats ($p < 0.001$), while stressed rats stroked at 5 cm/s spent significantly less time floating than stressed rats ($p < 0.001$). Stressed rats stroked at 30 cm/s spent significantly more time floating than stressed rats stroked at 5 cm/s ($p < 0.001$) (Figure 4A). Conversely, stressed rats spent less time in climbing behavior than non-stressed animals ($p < 0.001$), while the stressed rats that were stroked at 5 cm/s spent a more time in climbing behavior to stressed animals ($p < 0.001$) (Figure 4B). Stressed rats spent a comparable time in climbing behavior to stressed animals stroked at 30 cm/s ($p = 0.84$) (Figure 4B). Stroked rats to 5 cm/s and 30 cm/s spent more time in swimming than non-stressed and stressed rats ($p < 0.001$) (Figure 4C).

Social interaction.

Figure 5 shows that social behavior in the social preference-avoidance test was affected in the rats that were exposed to CMS and stroking stimulation. CMS protocol and stroking stimulation did not affect time spent in the interaction zone in the habituation phase ($p = 0.13$) (Figure 5A). In the social interaction phase, rats exposed to CMS spent less time in the interaction zone than non-stress rats ($p < 0.001$), while stroking stimulation did not affect time spent in the interaction zone by stressed rats ($p = 0.29$) (Figure 5B). Interestingly, stressed rats spent more time in the corners of the open field than non-stressed ($p < 0.001$), while rats exposed to CMS and stroked at 5 cm/s or 30 cm/s spent less time in the corners compared with stressed rats ($p < 0.001$) (Figure 5C). As in the results obtained for percentage of social interaction, CMS decreased the percentage time spent in social interaction compared to non-stressed rats ($p < 0.001$), while stroking stimulation at either velocity had no effect ($p = 0.28$) (Figure 5D).

DISCUSSION

In the present study, adult rats exposed daily for two weeks to a CMS protocol showed classically reported physiological and behavioral markers of stress and depressive-like behaviors (Moreau, 1997; Cerqueira *et al.*, 2007; Castro *et al.*, 2012; Ortiz & Conrad, 2018). Physiologically, in Experiment 1, rats exposed to two weeks of CMS showed significantly elevated corticosterone responses 15 minutes after acute exposure to a forced swim and, in Experiment 2, were of significantly lower body weight than non-stressed rats. Behaviorally, in the absence of any general changes in locomotor activity, rats exposed to two weeks of CMS spent significantly less time in the center of the open field as well as in the open arms of an elevated plus-maze than non-stressed rats. Also, during a forced swim test, they spent significantly more time floating and less time climbing. Finally, in a social interaction test following CMS rats spent less time in the interaction zone and more time in the corner than non-stressed rats. These findings are in line with several recent reports that CMS protocols lasting 10-14 days induce significant physiological and behavioral markers of chronic distress (Vyas *et al.*, 2002; Castro *et al.*, 2012).

Consistent with the previously reported stress buffering effects of gentle handling and tactile stimulation (Aulich *et al.*, 1974; Costa *et al.*, 2020), another group of rats exposed to the same CMS protocol but which received 10 minutes of gentle, CLTM optimal velocity (5 cm/s), head to tail stroking on their dorsum immediately prior to the daily stressor showed fewer physiological and behavioral markers of chronic distress. That is, while in Experiment 1, they still had significantly lower body weights than non-stressed rats, they did not show a significant elevation in corticosterone levels 15 minutes after the acute forced swim test. In fact, they did not differ from non-stressed controls. Notably, in line with our hypothesis, the stress buffering effects of daily stroking were velocity specific in that stroking at faster non-CLTM optimal velocity (30 cm/s) had no such buffering effect on endocrine reactions to the forced swim test. The same differential effects of stroking velocity were seen behaviorally, in Experiment 2, where in the absence of any changes in general locomotor activity in either group, the rats receiving CLTM optimal velocity stroking spent significantly more time in the open arms of the elevated plus-maze and less time floating, and more time climbing, in the forced swim test than non-CLTM optimal (30 cm/s) stroked rats.

While consistent with previous studies, CMS significantly reduced interactions in the social interaction test, here the effects were not buffered by stroking at either CLTM optimal or non-optimal velocities. However, more detailed analysis of the behavior of both groups of stroked rats indicates, indicative of a general anxiolytic effect, they spent more time in the center and less time in the corners of the arena than stressed rats. Social behavior cannot be reduced purely to physical contact. Social interaction can also be mediated via other sensory systems, such as hearing, smell, and vision (Nicol, 1995). Therefore, it is plausible that both groups of stroked rats were engaged in prosocial behavior using other sensory systems and not just physical contact. Electrophysiological recordings have previously identified elevations in gamma-band power within the nucleus accumbens of rats during spontaneous social interaction whereas rats exposed to CMS showed no such changes in neural activity (Iturra-Mena *et al.*, 2019) . Such neural markers could be used in future studies to further investigate the effects of stroking touch on spontaneous social interaction.

It is important to note that the low intensity cutaneous stimulation, such as gentle stroking touch, will result in the activation of all classes of cutaneous low threshold mechanosensitive afferent fibers, not just C-LTMs. For example, D-hair afferent fibers, which are Aδ fibres with intermediate conduction velocities, innervate all awl/auchene and zigzag hairs in rodents (Li *et al.*, 2011). They are rapidly adapting receptors that fire in response to hair movement (Li *et al.*, 2011; Lechner & Lewin, 2013). D-hair afferents are extremely sensitive to low mechanical forces and respond more vigorously to low velocity mechanical stimuli than large diameter, rapidly conducting Aβ fibers innervating hair follicles (Brown & Iggo, 1967; Milenkovic *et al.*, 2008; Lechner & Lewin, 2013). Thus, stroking stimuli at low and intermediate velocities will preferentially activate both C-LTMs and low threshold Aδ hair afferents. However, similar to Aβ afferent fibers, the firing rates of Aδ hair afferents increase with increasing velocity of the applied stimulus and do not show the classical velocity inverted-U shaped firing properties exhibited by CLTMs (Milenkovic *et al.*, 2008; Löken *et al.*, 2009; Lechner & Lewin, 2013). Thus, the velocity specific nature of the stress buffering effects we report here add weight to the argument that C-LTM activation plays a causal role.

Taken together, the findings from the current study are consistent with previous reports that stroking touch can buffer against the negative physiological and behavioral effects of CMS (Boufleur *et al.*, 2013; Freitas *et al.*, 2015; Antoniazzi *et al.*, 2017; Costa *et al.*, 2020). However, they also

extend those findings by providing insight into the underlying neurobiological mechanisms. That is, that the specific activation of a class of cutaneous mechanosensory afferent, for which the preferred stimulus is dynamic stroking at between 1-10 cm/s, mediates the observed effects. Our control condition, 30 cm/s strokes, has been widely used in human behavioral, psychophysical and neurophysiological studies (Liljencrantz *et al.*, 2014; Macefield *et al.*, 2014; Perini *et al.*, 2015; Pawling, Cannon, *et al.*, 2017; Pawling, Trotter, *et al.*, 2017; Haggarty *et al.*, 2020) based on the observation in single unit microneurography recordings that it activates CLTMs to a significantly lesser degree than stroking within their preferred range (Löken *et al.*, 2009; Ackerley *et al.*, 2014). The finding that CLTM activation buffers against some of the negative neuroendocrine and behavioral effects of repeated stress is consistent with theories of CLTM function which propose, given their response characteristics, central projections and behavioral effects, they evolved to signal the rewarding value of affiliative tactile interactions and contribute to the maintenance of homeostasis (Björnsdotter *et al.*, 2010; Morrison *et al.*, 2010; Walker & McGlone, 2013; Morrison, 2016; Walker, Trotter, Swaney, *et al.*, 2017).The present study was conducted in adult rats, while to date, most investigations of the stress buffering effects of touch have been conducted during the neonatal period. Thus, further work is needed to determine whether the CLTM velocity dependent stress buffering effects we report here also underpin the long-term stress resilience conferred by early life tactile stimulation. Developmental studies have reliably shown that maternal tactile stimulation is an important regulator of HPA axis development, and the beneficial effects are apparent across the lifespan, in part due to an upregulation of glucocorticoid receptor availability, particularly in the hippocampus (Liu *et al.*, 1997; Hellstrom *et al.*, 2012; van Hasselt *et al.*, 2012). In the absence of maternal care these beneficial effects are mimicked by stroking with a soft brush (Van Oers *et al.*, 1998; Gonzalez *et al.*, 2001; Hellstrom *et al.*, 2012). A previous study in neonate rats reported that daily tactile stimulation produced a rapid enhancement of glucocorticoid receptor gene expression, within just two days (Jutapakdeegul *et al.*, 2003). If a similar, rapid up-regulation occurred in adult rats, ensuring efficient termination of the stress response through negative feedback mechanisms (McEwen & Akil, 2020) that would help explain the resilience to the typical effects of CMS observed in the stroked rats reported here.

There are several limitations to the present study which warrant further investigation. For example, CLTM optimal stroking did not buffer against all deleterious effects of the CMS protocol. Stroked rats still showed significantly lower body weight and reduced social interaction in comparison to non-stressed rats. This could be because the present intervention was not optimized or may reflect the fact that CLTM activation buffers against some aspects of CMS but not others. Here we used a 10-minute daily stroking intervention as in previous studies, in adult rodents and human infants, we have found it to induce acute relaxation effects on physiology and behavior (Walker, Trotter, Swaney, *et al.*, 2017; Manzotti *et al.*, 2019; Van Puyvelde *et al*., 2019). The lack of effect of tactile intervention on weight gain is consistent with a previous report in adult CMS exposed rats (Costa *et al.*, 2020), where a shorter daily dose, but longer total duration of tactile intervention was administered. Resilient animals still respond physiologically to stress, they just adapt more quickly to stressors (Cathomas et. al., 2019). The neuroendocrine systems involved in stress susceptibility and resilience consume energy and that energy cost may explain the lower body weights in all the rats exposed to CMS, compared to non-stressed rats. However, the present findings are inconsistent with previous developmental studies which have reported increased weight gain in maternally separated rats pups and premature infants following repeated massage and stroking stimulation (Schanberg & Field, 1987). Thus, further work is needed to optimize our own tactile intervention in terms of frequency and duration. Also, in the present study, we concentrated on stroking the back as previous genetic visualization (Liu *et al.*, 2007) and behavioral (Walker, Trotter, Woods, *et al.*, 2017) studies indicate CLTMs innervate this area most densely. However, CLTMs do show fatigue, represented by a reduced firing rate to repeated tactile stimuli (Vallbo *et al.*, 1999). Therefore, comparison of the physiological effects of the same period of CLTM targeted touch delivered to a single versus a range of body sites would be insightful.

In conclusion, in highly social species affiliative touch plays a salient role in intimate relationships, with important neurodevelopmental, emotional, and social consequences (Feldman & Eidelman, 2007; Dunbar, 2010; Walker, 2010; Walker & McGlone, 2013; Sullivan & Perry, 2015). While the neurobiological basis of touch's stress buffering effects remains to be fully elucidated, in the present study we show, for the first time, that 10 minutes of stroking touch, targeted to activate a specific class of unmyelinated, low threshold mechanoreceptor found in the hairy skin of mammals,

buffers adult rats from many of the anxiogenic and anhedonic effects CMS exposure. Future work will investigate the neural and neurochemical basis of the observed effects.

Acknowledgments

This research was supported by Anillo de Ciencia y Tecnología, Programa PIA, CONICYT (Grant Number ACT1403) to Alexies Dagnino-Subiabre.

Abbreviations

ANOVA: Analysis of variance; BLA: Basolateral amygdala; BNST: Bed-nucleus of the stria terminalis; CLTMs: Class of low-threshold c-fiber mechanoreceptors; CMS: Chronic unpredictable mild stress ; CORT: Corticosterone; CRH: Corticotropin releasing hormone; EPM: Elevated plus-maze; FST: Forced swim test; HPA: Hypotalamic pituitary adrenal; NTS: Nucleus of the solitary tract; OF: Open field; PI: Posterior insula; PND: Post-natal day; PVN: Paraventricular nucleus of the hypothalamus; SEM: Standard error of the mean; SI: Social interaction; SNS: Sympathetic nervous system; SPT: Sucrose preference test

Competing Interests

The authors declare no conflict of interest.

Author Contributions

A.D-S. and F.P.M. designed the study. A.C, V.P-S., and W.E-D. did the experiments and analyzed the data. A.D-S, S.W., and F.P.M wrote the article.

Data Accessibility

All data presented in this manuscript can be accessed by contacting the corresponding author.

References

Abraira, V.E., Watanabe, M., Dymecki, S.M., Chirila, A.M., Springel, M.W., Toliver, A.A., Zimmerman, A.L., Orefice, L.L., Bai, L., Song, B.J., Bashista, K.A., O'Neill, T.G., Zhuo, J., Tsan, C., Hoynoski, J., & Ginty, D.D. (2017) The Cellular and Synaptic Architecture of the Mechanosensory Dorsal Horn. *Cell*, **168**, 295-310.e19.

Ackerley, R., Backlund Wasling, H., Liljencrantz, J., Olausson, H., Johnson, R.D., & Wessberg, J. (2014) Human C-Tactile Afferents Are Tuned to the Temperature of a Skin-Stroking Caress. *J. Neurosci.*, **34**, 2879–2883.

Al-Khater, K.M. & Todd, A.J. (2009) Collateral projections of neurons in laminae I, III, and IV of rat spinal cord to thalamus, periaqueductal gray matter, and lateral parabrachial area. *J. Comp. Neurol.*, **515**, 629–646.

Alden, M., Besson, J. -M, & Bernard, J. -F (1994) Organization of the efferent projections from the pontine parabrachial area to the bed nucleus of the stria terminalis and neighboring regions: A PHA-L study in the rat. *J. Comp. Neurol.*, **341**, 289–314.

Andrew, D. (2010) Quantitative characterization of low-threshold mechanoreceptor inputs to lamina I spinoparabrachial neurons in the rat. *J. Physiol.*, **588**, 117–124.

Antoniazzi, C.T.D., Metz, V.G., Roversi, K., Freitas, D.L., Vey, L.T., Dias, V.T., Segat, H.J., Duarte, M.M.M.F., & Burger, M.E. (2017) Tactile stimulation during different developmental periods modifies hippocampal BDNF and GR, affecting memory and behavior in adult rats. *Hippocampus*, **27**, 210–220.

Araki, T., Ito, K., Kurosawa, M., & Sato, A. (1984) Responses of adrenal sympathetic nerve activity and catecholamine secretion to cutaneous stimulation in anesthetized rats. *Neuroscience*, **12**, 289–299.

Aulich, D., Spielhofen, J., & Raaijmakers, W.G.M. (1974) The influence of adult handling and social isolation on dark preference in albino rats. *Anim. Behav.*, **22**, 987–990.

Bernard, J. -F, Alden, M., & Besson, J. -M (1993) The organization of the efferent projections from the pontine parabrachial area to the amygdaloid complex: A phaseolus vulgaris leucoagglutinin (PHA-L) study in the rat. *J. Comp. Neurol.*, **329**, 201–229.

Bessou, P., Burgess, P.R., Perl, E.R., & Taylor, C.B. (1971) Dynamic properties of mechanoreceptors with unmyelinated (C) fibers. *J. Neurophysiol.*, **34**, 116–131.

Bester, H., Besson, J.M., & Bernard, J.F. (1997) Organization of efferent projections from the parabrachial area

to the hypothalamus: A Phaseolus vulgaris-leucoagglutinin study in the rat. *J. Comp. Neurol.*, **383**, 245–281.

Björnsdotter, M., Morrison, I., & Olausson, H. (2010) Feeling good: On the role of C fiber mediated touch in interoception. *Exp. Brain Res.*, **207**, 149–155.

Boufleur, N., Antoniazzi, C.T.D., Pase, C.S., Benvegnú, D.M., Dias, V.T., Segat, H.J., Roversi, K., Roversi, K., Nora, M.D., Koakoskia, G., Rosa, J.G., Barcellos, L.J.G., & Bürger, M.E. (2013) Neonatal handling prevents anxiety-like symptoms in rats exposed to chronic mild stress: Behavioral and oxidative parameters. *Stress*, **16**, 321–330.

Brown, B.Y.A.G. & Iggo, A. (1967) From the Department of Veterinary Physiology , University of Edinburgh 707–733.

Caldji, C., Tannenbaum, B., Sharma, S., Francis, D., Plotsky, P.M., & Meaney, M.J. (1998) Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. *Proc. Natl. Acad. Sci. U. S. A.*, **95**, 5335–5340.

Castro, J.E., Diessler, S., Varea, E., Márquez, C., Larsen, M.H., Cordero, M.I., & Sandi, C. (2012) Personality traits in rats predict vulnerability and resilience to developing stress-induced depression-like behaviors, HPA axis hyper-reactivity and brain changes in pERK1/2 activity. *Psychoneuroendocrinology*, **37**, 1209–1223.

Cerqueira, J.J., Mailliet, F., Almeida, O.F.X., Jay, T.M., & Sousa, N. (2007) The prefrontal cortex as a key target of the maladaptive response to stress. *J. Neurosci.*, **27**, 2781–2787.

Champagne, F.A. (2008) Epigenetic mechanisms and the transgenerational effects of maternal care. *Front. Neuroendocrinol.*, **29**, 386–397.

Champagne, F.A., Francis, D.D., Mar, A., & Meaney, M.J. (2003) Variations in maternal care in the rat as a mediating influence for the effects of environment on development. In *Physiology and Behavior*. Elsevier Inc., pp. 359–371.

Champagne, F.A. & Meaney, M.J. (2007) Transgenerational Effects of Social Environment on Variations in Maternal Care and Behavioral Response to Novelty. *Behav. Neurosci.*, **121**, 1353–1363.

Costa, R., Tamascia, M.L., Sanches, A., Moreira, R.P., Cunha, T.S., Nogueira, M.D., Casarini, D.E., & Marcondes, F.K. (2020) Tactile stimulation of adult rats modulates hormonal responses, depression-like behaviors, and memory impairment induced by chronic mild stress: Role of angiotensin II. *Behav. Brain*

Res., **379**.

Craig, a. D. (2003) Interoception: The sense of the physiological condition of the body. *Curr. Opin. Neurobiol.*, **13**, 500–505.

Ditzen, B., Neumann, I.D., Bodenmann, G., von Dawans, B., Turner, R.A., Ehlert, U., & Heinrichs, M. (2007) Effects of different kinds of couple interaction on cortisol and heart rate responses to stress in women. *Psychoneuroendocrinology*, **32**, 565–574.

Douglas, W.W. & Ritchie, J.M. (1957) Non-medullated fibres in the saphenous nerve which signal touch. *J. Physiol.*, **139**, 385–399.

Dunbar, R.I.M. (2010) The social role of touch in humans and primates: Behavioural function and neurobiological mechanisms. *Neurosci. Biobehav. Rev.*, **34**, 260–268.

Essick, G.K., James, A., & McGlone, F.P. (1999) Psychophysical assessment of the affective components of non-painful touch. *Neuroreport*, **10**, 2083–2087.

Essick, G.K., McGlone, F., Dancer, C., Fabricant, D., Ragin, Y., Phillips, N., Jones, T., & Guest, S. (2010) Quantitative assessment of pleasant touch. *Neurosci. Biobehav. Rev.*, **34**, 192–203.

Feldman, R. & Eidelman, A.I. (2007) Skin-to-skin contact (Kangaroo Care) accelerates autonomic and neurobehavioural maturation in preterm infants. *Dev. Med. Child Neurol.*, **45**, 274–281.

Francis, T.C., Chandra, R., Friend, D.M., Finkel, E., Dayrit, G., Miranda, J., Brooks, J.M., Iñiguez, S.D., O'Donnell, P., Kravitz, A., & Lobo, M.K. (2015) Nucleus accumbens medium spiny neuron subtypes mediate depression-related outcomes to social defeat stress. *Biol. Psychiatry*, **77**, 212–222.

Franklin, T.B., Saab, B.J., & Mansuy, I.M. (2012) Neural Mechanisms of Stress Resilience and Vulnerability. *Neuron*,.

Freitas, D., Antoniazzi, C.T.D., Segat, H.J., Metz, V.G., Vey, L.T., Barcelos, R.C.S., Duarte, T., Duarte, M.M.M.F., & Burger, M.E. (2015) Neonatal tactile stimulation decreases depression-like and anxiety-like behaviors and potentiates sertraline action in young rats. *Int. J. Dev. Neurosci.*, **47**, 192–197.

Gauriau, C. & Bernard, J.F. (2004) Posterior Triangular Thalamic Neurons Convey Nociceptive Messages to the Secondary Somatosensory and Insular Cortices in the Rat. *J. Neurosci.*, **24**, 752–761.

Gonzalez, A., Lovic, V., Ward, G.R., Wainwright, P.E., & Fleming, A.S. (2001) Intergenerational effects of complete maternal deprivation and replacement stimulation on maternal behavior and emotionality in female rats. *Dev. Psychobiol.*, **38**, 11–32.

Gordon, I., Voos, A.C., Bennett, R.H., Bolling, D.Z., Pelphrey, K.A., & Kaiser, M.D. (2013) Brain mechanisms for processing affective touch. *Hum. Brain Mapp.*, **34**, 914–922.

Haggarty, C.J., Malinowski, P., McGlone, F.P., & Walker, S.C. (2020) Autistic traits modulate cortical responses to affective but not discriminative touch. *Eur. J. Neurosci.*, ejn.14637.

Hellstrom, I.C., Dhir, S.K., Diorio, J.C., & Meaney, M.J. (2012) Maternal licking regulates hippocampal glucocorticoid receptor transcription through a thyroid hormone-serotonin-NGFI-A signalling cascade. *Philos. Trans. R. Soc. B Biol. Sci.*, **367**, 2495–2510.

Iggo, A. & Kornhuber, H.H. (1977) A quantitative study of C-mechanoreceptors in hairy skin of the cat. *J. Physiol.*, **271**, 549–565.

Iturra-Mena, A.M., Aguilar-Rivera, M., Arriagada-Solimano, M., Pérez-Valenzuela, C., Fuentealba, P., & Dagnino-Subiabre, A. (2019) Impact of stress on gamma oscillations in the rat nucleus accumbens during spontaneous social interaction. *Front. Behav. Neurosci.*, **13**, 151.

Jacinto, L.R., Cerqueira, J.J., & Sousa, N. (2016) Patterns of theta activity in limbic anxiety circuit preceding exploratory behavior in approach-avoidance conflict. *Front. Behav. Neurosci.*, **10**.

Jutapakdeegul, N., Stefano, O., Govitrapong, P., & Kotchabhakdi, N. (2003) Postnatal Touch Stimulation Acutely Alters Corticosterone Levels and Glucocorticoid Receptor Gene Expression in the Neonatal Rat. *Dev. Neurosci.*, **25**, 26–33.

Kumazawa, T. & Perl, E.R. (1977) Primate cutaneous sensory units with unmyelinated (C) afferent fibers. *J. Neurophysiol.*, **40**, 1325–1338.

Lechner, S.G. & Lewin, G.R. (2013) Hairy sensation. *Physiology (Bethesda).*, **28**, 142–150.

Li, L., Rutlin, M., Abraira, V.E., Cassidy, C., Kus, L., Gong, S., Jankowski, M.P., Luo, W., Heintz, N., Koerber, H.R., Woodbury, C.J., & Ginty, D.D. (2011) The functional organization of cutaneous low-threshold mechanosensory neurons. *Cell*, **147**, 1615–1627.

Liljencrantz, J., Marshall, A., Ackerley, R., & Olausson, H. (2014) Discriminative and affective touch in human experimental tactile allodynia. *Neurosci. Lett.*, **563**, 75–79.

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., Freedman, A., Sharma, S., Pearson, D., Plotsky, P.M., & Meaney, M.J. (1997) Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science (80-.).*, **277**, 1659–1662.

Liu, Q., Vrontou, S., Rice, F.L., Zylka, M.J., Dong, X., & Anderson, D.J. (2007) Molecular genetic

visualization of a rare subset of unmyelinated sensory neurons that may detect gentle touch. *Nat. Neurosci.*, **10**, 946–948.

Löken, L.S., Wessberg, J., Morrison, I., McGlone, F., & Olausson, H. (2009) Coding of pleasant touch by unmyelinated afferents in humans. *Nat. Neurosci.*, **12**, 547–548.

Lu, Y. & Perl, E.R. (2005) Modular organization of excitatory circuits between neurons of the spinal superficial dorsal horn (laminae I and II). *J. Neurosci.*, **25**, 3900–3907.

Lund, I., Lundeberg, T., Kurosawa, M., & Uvnäs-Moberg, K. (1999) Sensory stimulation (massage) reduces blood pressure in unanaesthetized rats. *J. Auton. Nerv. Syst.*, **78**, 30–37.

Lynn, B. & Carpenter, S.E. (1982) Primary afferent units from the hairy skin of the rat hind limb. *Brain Res.*, **238**, 29–43.

Macefield, V.G., Norcliffe-Kaufmann, L., L??ken, L., Axelrod, F.B., & Kaufmann, H. (2014) Disturbances in affective touch in hereditary sensory & autonomic neuropathy type III. *Int. J. Psychophysiol.*, **93**, 56–61.

Manzotti, A., Cerritelli, F., Esteves, J.E., Lista, G., Lombardi, E., La Rocca, S., Gallace, A., McGlone, F.P., & Walker, S.C. (2019) Dynamic touch reduces physiological arousal in preterm infants: A role for c-tactile afferents? *Dev. Cogn. Neurosci.*, **39**.

Marshall, A.G., Sharma, M.L., Marley, K., Olausson, H., & McGlone, F.P. (2019) Spinal signalling of c-fiber mediated pleasant touch in humans. *Elife*, **8**.

Maruyama, K., Shimoju, R., Ohkubo, M., Maruyama, H., & Kurosawa, M. (2012) Tactile skin stimulation increases dopamine release in the nucleus accumbens in rats. *J. Physiol. Sci.*, **62**, 259–266.

McEwen, B.S. (1998) Stress, Adaptation, and Disease: Allostasis and Allostatic Load. *Ann. N. Y. Acad. Sci.*, **840**, 33–44.

McEwen, B.S. & Akil, H. (2020) Revisiting the stress concept: Implications for affective disorders. *J. Neurosci.*, **40**, 12–21.

Mcglone, F., Olausson, H., Boyle, J. a., Jones-Gotman, M., Dancer, C., Guest, S., & Essick, G. (2012) Touching and feeling: Differences in pleasant touch processing between glabrous and hairy skin in humans. *Eur. J. Neurosci.*, **35**, 1782–1788.

McGlone, F., Wessberg, J., & Olausson, H. (2014) Discriminative and Affective Touch: Sensing and Feeling. *Neuron*, **82**, 737–755.

Meaney, M.J. (2001) Maternal Care, Gene Expression, and the Transmission of Individual Differences in

Stress Reactivity Across Generations. *Annu. Rev. Neurosci.*, **24**, 1161–1192.

Milenkovic, N., Wetzel, C., Moshourab, R., & Lewin, G.R. (2008) Speed and temperature dependences of mechanotransduction in afferent fibers recorded from the mouse saphenous nerve. *J. Neurophysiol.*, **100**, 2771–2783.

Moreau, J.L. (1997) [Validation of an animal model of anhedonia, a major symptom of depression]. *Encephale.*, **23**, 280–289.

Morrison, I. (2016) Keep Calm and Cuddle on: Social Touch as a Stress Buffer. *Adapt. Hum. Behav. Physiol.*, 344–362.

Morrison, I., Löken, L.S., & Olausson, H. (2010) The skin as a social organ. *Exp. Brain Res.*, **204**, 305–314.

Nicol, C.J. (1995) The social transmission of information and behaviour. *Appl. Anim. Behav. Sci.*, **44**, 79–98.

Nordin, M. (1990). Low-threshold mechanoreceptive and nociceptive units with unmyelinated (C) fibres in the human supraorbital nerve. J. Physiol. 426, 229–240.

Nummenmaa, L., Tuominen, L., Dunbar, R., Hirvonen, J., Manninen, S., Arponen, E., Machin, A., Hari, R., Jääskeläinen, I.P., & Sams, M. (2016) Social touch modulates endogenous μ-opioid system activity in humans. *Neuroimage*, **138**, 242–247.

Olausson, H., Lamarre, Y., Backlund, H., Morin, C., Wallin, B.G., Starck, G., Ekholm, S., Strigo, I., Worsley, K., Vallbo, a B., & Bushnell, M.C. (2002) Unmyelinated tactile afferents signal touch and project to insular cortex. *Nat. Neurosci.*, **5**, 900–904.

Olausson, H., Wessberg, J., Morrison, I., McGlone, F., & Vallbo, Å. (2010) The neurophysiology of unmyelinated tactile afferents. *Neurosci. Biobehav. Rev.*, **34**, 185–191.

Ortiz, J.B. & Conrad, C.D. (2018) The impact from the aftermath of chronic stress on hippocampal structure and function: Is there a recovery? *Front. Neuroendocrinol.*,.

Parker, K.J., Buckmaster, C.L., Sundlass, K., Schatzberg, A.F., & Lyons, D.M. (2006) Maternal mediation, stress inoculation, and the development of neuroendocrine stress resistance in primates. *Proc. Natl. Acad. Sci. U. S. A.*, **103**, 3000–3005.

Pawling, R., Cannon, P.R., McGlone, F.P., & Walker, S.C. (2017) C-tactile afferent stimulating touch carries a positive affective value. *PLoS One*, **12**.

Pawling, R., Trotter, P.D., McGlone, F.P., & Walker, S.C. (2017) A positive touch: C-tactile afferent targeted skin stimulation carries an appetitive motivational value. *Biol. Psychol.*, **129**.

Perini, I., Morrison, I., & Olausson, H. (2015) Seeking pleasant touch: neural correlates of behavioral preferences for skin stroking. *Front. Behav. Neurosci.*, **9**, 1–9.

Polgár, E., Wright, L.L., & Todd, A.J. (2010) A quantitative study of brainstem projections from lamina I neurons in the cervical and lumbar enlargement of the rat. *Brain Res.*, **1308**, 58–67.

Schanberg, S.M. & Field, T.M. (1987) Sensory deprivation stress and supplemental stimulation in the rat pup and preterm human neonate. *Child Dev.*, **58**, 1431–1447.

Stock, S. & Uvnäs-Moberg, K. (1988) Increased plasma levels of oxytocin in response to afferent electrical stimulation of the sciatic and vagal nerves and in response to touch and pinch in anaesthetized rats. *Acta Physiol. Scand.*, **132**, 29–34.

Strigo, I.A. & Craig, A.D. (2016) Interoception, homeostatic emotions and sympathovagal balance. *Philos. Trans. R. Soc. B Biol. Sci.*, **371**.

Sullivan, R.M. & Perry, R.E. (2015) Infants : Lessons from Animal Models **10**, 500–511.

Uvnäs-Moberg, K., Alster, P., Lund, I., Lundeberg, T., Kurosawa, M., & Ahlenius, S. (1996) Stroking of the abdomen causes decreased locomotor activity in conscious male rats. *Physiol. Behav.*, **60**, 1409–1411.

Uvnäs-Moberg, K., Handlin, L., & Petersson, M. (2014) Self-soothing behaviors with particular reference to oxytocin release induced by non-noxious sensory stimulation. *Front. Psychol.*, **5**, 1529.

Vallbo, Å.B., Olausson, H., & Wessberg, J. (1999) Unmyelinated afferents constitute a second system coding tactile stimuli of the human hairy skin. *J. Neurophysiol.*, **81**, 2753–2763.

van Hasselt, F.N., Cornelisse, S., Yuan Zhang, T., Meaney, M.J., Velzing, E.H., Krugers, H.J., & Joëls, M. (2012) Adult hippocampal glucocorticoid receptor expression and dentate synaptic plasticity correlate with maternal care received by individuals early in life. *Hippocampus*, **22**, 255–266.

Van Oers, H.J.J., De Kloet, E.R., Whelan, T., & Levine, S. (1998) Maternal deprivation effect on the infant's neural stress markers is reversed by tactile stimulation and feeding but not by suppressing corticosterone. *J. Neurosci.*, **18**, 10171–10179.

Van Puyvelde M., Collette L., Gorissen A.S., Pattyn N., McGlone F. (2019) Infants Autonomic Cardio-Respiratory Responses to Nurturing Stroking Touch Delivered by the Mother or the Father. *Front Physiol*., 10:1117.

Vannorsdall, T., Dahlquist, L., Pendley, J.S., & Power, T. (2004) The relation between nonessential touch and children's distress during lumbar punctures. *Child. Heal. Care*, **33**, 299–315.

Vrontou, S., Wong, A.M., Rau, K.K., Koerber, H.R., & Anderson, D.J. (2013) Genetic identification of C fibres that detect massage-like stroking of hairy skin in vivo. *Nature*, **493**, 669–673.

Vyas, A., Mitra, R., Shankaranarayana Rao, B.S., & Chattarji, S. (2002) Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. *J. Neurosci.*, **22**, 6810–6818.

Walker, C.D. (2010) Maternal touch and feed as critical regulators of behavioral and stress responses in the offspring. *Dev. Psychobiol.*, **52**, 638–650.

Walker, S.C. & McGlone, F.P. (2013) The social brain: Neurobiological basis of affiliative behaviours and psychological well-being. *Neuropeptides*, **47**.

Walker, S.C., Trotter, P.D., Swaney, W.T., Marshall, A., & Mcglone, F.P. (2017) C-tactile afferents: Cutaneous mediators of oxytocin release during affiliative tactile interactions? *Neuropeptides*, **64**, 27–38.

Walker, S.C., Trotter, P.D., Woods, A., & McGlone, F. (2017) Vicarious ratings of social touch reflect the anatomical distribution & velocity tuning of C-tactile afferents: A hedonic homunculus? *Behav. Brain Res.*, **320**.

Wercberger, R. & Basbaum, A.I. (2019) Spinal cord projection neurons: a superficial, and also deep analysis. *Curr. Opin. Physiol.*, **11**, 109–115.

Zoicas, I. & Neumann, I.D. (2016) Maternal separation facilitates extinction of social fear in adult male mice. *Behav. Brain Res.*, **297**, 323–328.

Zotterman, Y. (1939) Touch, pain and tickling: an electro-physiological investigation on cutaneous sensory nerves. *J. Physiol.*, **95**, 1–28.

Figure Legends

Scheme 1: Experimental design. In both experiments, rats were divided into 4 groups (Non-Stress, Stress, Stress + 5 cm/s stroking and Stress + 30 cm/s stroking). From PND 70, chronic unpredictable mild stress (CMS) was applied for fourteen consecutive days. The tactile stimulation procedure was applied for ten minutes daily throughout this period, immediately prior to exposure to the daily stressor. In Experiment 1, one day after completion of the CMS protocol, blood samples were taken immediately before, plus 15 and 90 minutes after a 1-minute forced swim at 19 ºC. These were analyzed to establish serum corticosterone (CORT) levels. In Experiment 2, one day after completion of the CMS protocol, locomotor activity was established in the open field (OF) and depression-like behaviors were evaluated using the Social Interaction-Avoidance test (SI). The following day, two days after completion of the CMS protocol, anxiety-like-behaviors were evaluated on an elevated plus-maze (EPM) and depression-like behaviors were evaluated using the FST.

Figure 1: Effects of CMS and stroking on stress markers. (A) There were significant differences in the body weight gain between groups. All groups of rats exposed to CMS gained significantly less weight between PND 70 to PND 84 than the Non-Stress group (*$p < 0.01$). Thus, stroking velocity did not affect body weight gain in stressed animals. (B) Serum concentrations of corticosterone in four groups of rats immediately before, 15 minutes and 90 minutes after a forced swim. There were significant elevations of corticosterone recorded 15 minutes after the FST. However, Stress rats had significantly higher levels than Non-Stress rats. The Stress + 5 cm/s group had corticosterone levels comparable to Non-Stress rats while the Stress + 30 cm/s group had significantly higher levels than the Non-Stress rats (*$p < 0.001$) and were comparable to the Stress group. Thus, stoking at 5 cm/s but not 30 cm/s enhanced the endocrine system's resilience to CMS.

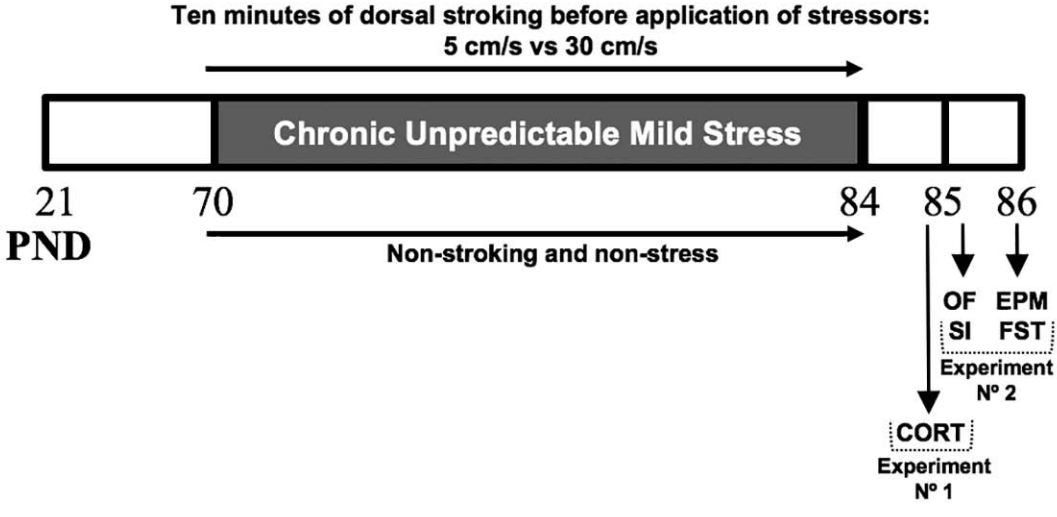
Figure 2: Locomotor activity. (A) Shows total distance travelled in the open field. There were no significant differences between any of the 4 groups. (B) Shows average speed of travel in the open field. Again, there were no significant differences between groups. Thus, exposure to CMS had no effect on locomotor activity. Figures C and D shows time spent in the center and border zone of the open field, respectively. Stressed rats spent significantly less time in the center zone and more time in

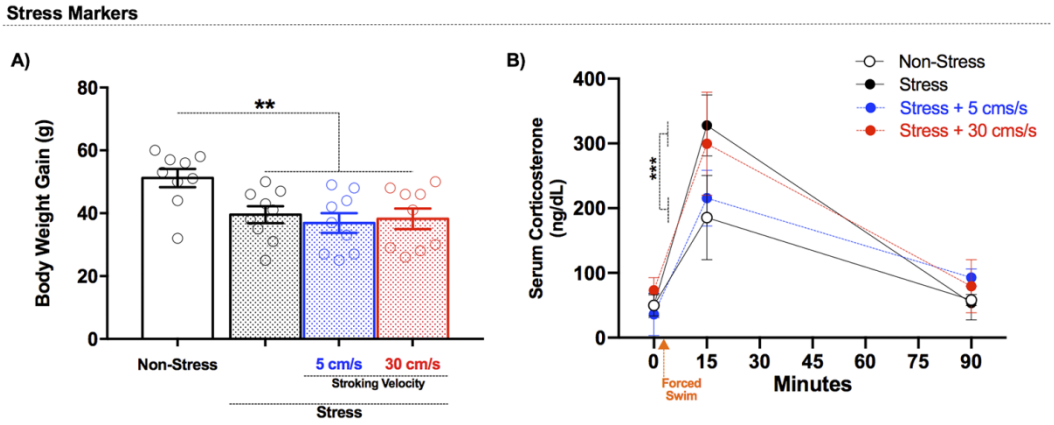
the border zone than the Non-Stress group (*$p < 0.05$). The Stress + 5 cm/s rats displayed a significantly more time in the center zone and less time the border zone than the Stress rats (*$p < 0.05$). Thus, stroking at 5 cm/s but not 30 cm/s mitigated the effect of CMS on center zone exploration.

Figure 3. Anxiety-like behaviors. (A) Time spent in the open arms of the elevated plus maze. Stress rats spent significantly less time in the open arms then the Non-Stress group (***$p < 0.001$). Stress + 5 cm/s rats spent significantly more time in the open arms than the Stress + 30 cm/s rats (***$p < 0.001$). (B) Ratio of open arm to total arm entries. The Non-Stress rats made a significantly higher ratio of open arm entries than the Stress rats (*$p < 0.01$). The Stress + 5 cm/s rats also displayed a significantly higher ratio of open arm entries than the Stress rats (*$p < 0.05$). Thus, stroking at 5 cm/s but not 30 cm/s mitigated the effect of CMS on open arm exploration.

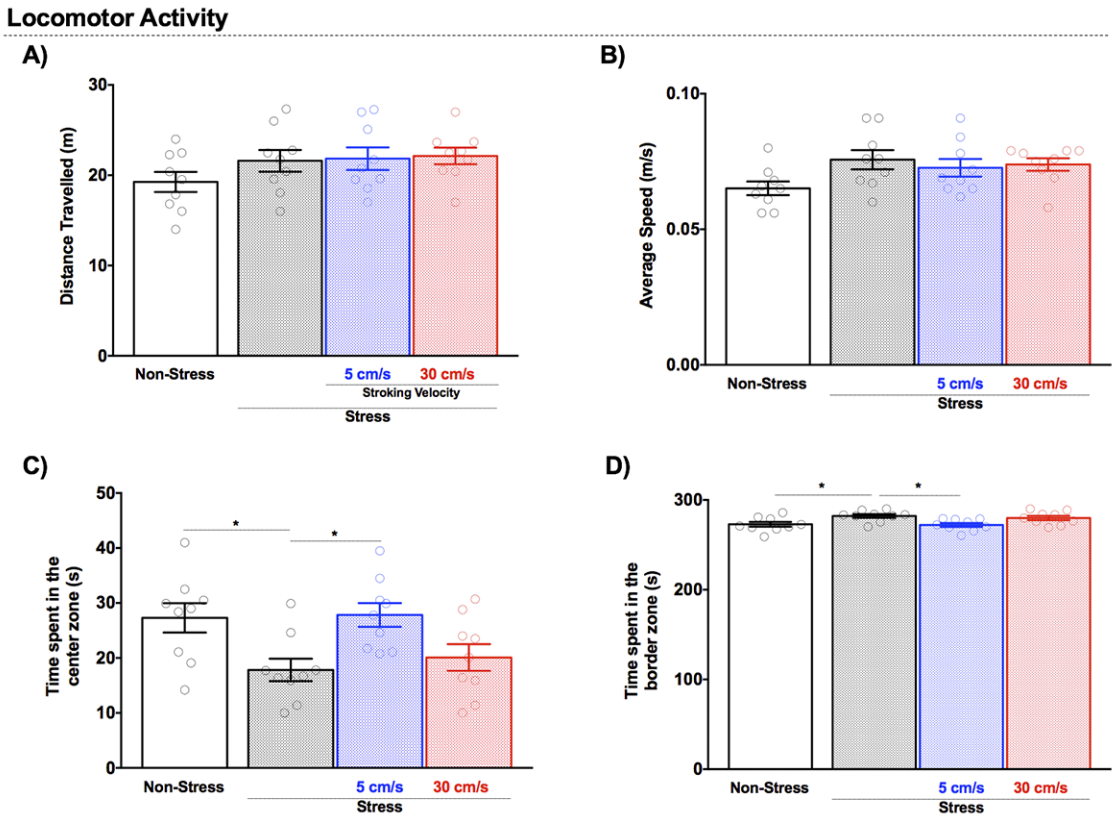
Figure 4. Floating (A), Climbing (B), and Swimming (C) behavior in the forced swim test. The Stress rats spent significantly more time floating and less time climbing than the Non-Stress rats (***$p < 0.001$). The Stress + 5 cm/s rats spent significantly less time floating and more time climbing than the Stress + 30 cm/s rats (***$p < 0.001$). Both groups of stroked rats (Stress + 5 cm/s and Stress + 30 cm/s) spent significantly more time swimming than Non-Stress and Stress rats (***$p < 0.001$). Stroking at 5 cm/s mitigated the effects of CMS on depressive-like behavior.

Figure 5. Social Preference-Avoidance test results for the four groups of rats. (A) Shows time spent in the interaction zone in the habituation phase. There were no significant differences between any of the 4 groups. (B) Non-Stress rats spent significantly more time engaged in social interaction than any of the groups of CMS exposed rats. (C) Stress rats spent significantly more time in the corner of the arena than either the Non-Stress rats or either group of stroked rats. (D) The Non-Stress group spent a significantly higher percentage of the total test time in social interaction than any of the three CMS exposed groups (Stress, Stress + 5 cm/s & Stress + 30 cm/s) (***$p < 0.001$). Thus, CMS significantly decreased social interaction and stroking did not mitigate this effect.

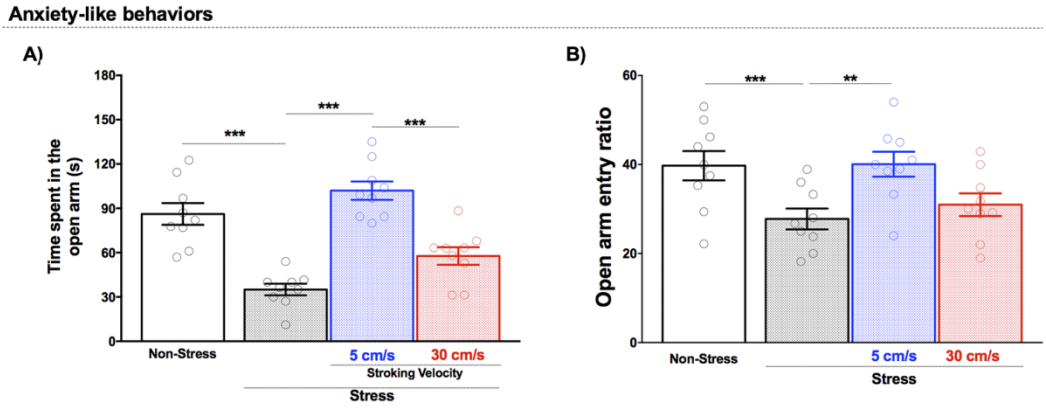

Ten minutes of dorsal stroking before application of stressors:
5 cm/s vs 30 cm/s
Chronic Unpredictable Mild Stress
21
PND
70
84
85
86
Non-stroking and non-stress
OF
SI
EPM
FST
Experiment
Nº 2
CORT
Experiment
Nº 1



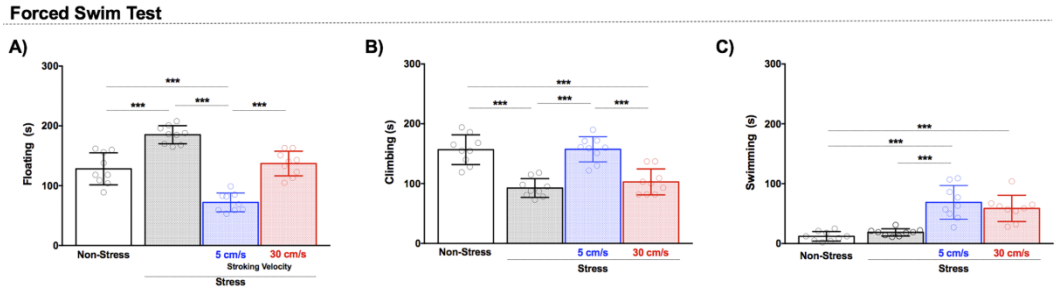
ejn_14951_f1.tif



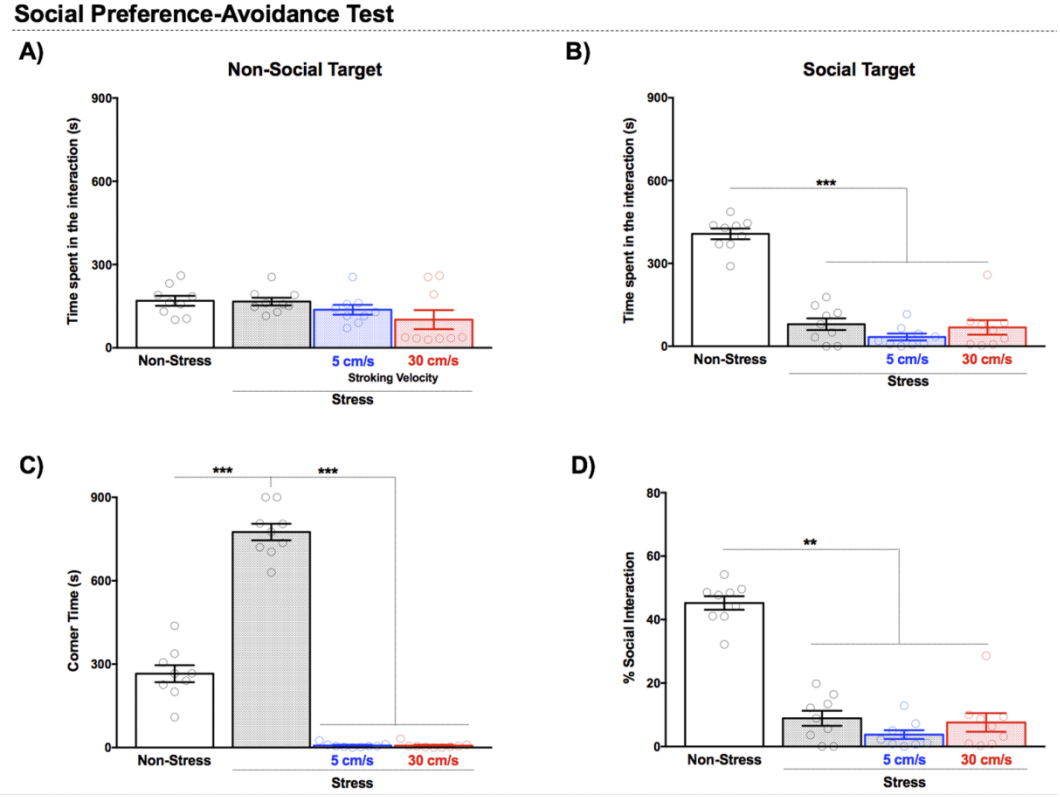
ejn_14951_f2.tif



ejn_14951_f3.tif



ejn_14951_f4.tif



ejn_14951_f5.tif