Brain Structure and Function (2019) 224:643–659
https://doi.org/10.1007/s00429-018-1789-8

ORIGINAL ARTICLE

# Adolescent social instability stress alters markers of synaptic plasticity and dendritic structure in the medial amygdala and lateral septum in male rats

**Travis E. Hodges**[1] · **Emma L. Louth**[2] · **Craig D. C. Bailey**[2] · **Cheryl M. McCormick**[1,3]

Received: 19 April 2018 / Accepted: 3 November 2018 / Published online: 23 November 2018

## Abstract

Much evidence indicates that experiences in adolescence can alter the development of social behaviour. We previously demonstrated that male rats exposed to social instability stress in adolescence (SS; 1 h isolation and return to an unfamiliar cagemate daily from postnatal day [PND] 30–45) had reduced social interaction, impaired social recognition, reduced sexual performance, and increased aggression in competition for food reward compared with non-stressed control (CTL) rats. Here, we investigated whether SS affects stellate neuron morphology using the Golgi–Cox method and several markers of synaptic plasticity using western blotting in the medial amygdala (MeA) and lateral septum (LS), sites involved in social behaviour. On PND 46, 24 h after the last stress exposure, SS rats had increased dendritic arborisation, a greater number of dendrite terminals, and a higher average dendrite branch order in the anterodorsal MeA compared with CTL rats. SS rats had reduced dendritic arborization and a reduced total length of dendrite matter in the anteroventral MeA and a reduced number of dendrite terminals in the posterodorsal MeA compared with CTL rats. Moreover, SS rats had a reduced number of dendritic spines in the dorsal LS compared with CTL rats. SS rats had less synaptophysin in the MeA and more CaMKII in the LS than did CTL rats, and did not differ in spinophilin, PSD95, or glucocorticoid receptor protein expression in the MeA and LS. We discuss how changes in neural structure and in markers of synaptic plasticity the MeA and LS of adolescent SS rats compared with CTL rats may underlie their differences in social behaviour.

**Keywords** Adolescence · Social instability · Dendritic arborisation · Synaptic plasticity · Medial amygdala · Lateral septum

## Introduction

Social experience during the adolescent period is crucial for the ability to display appropriate social behaviour in the future (reviewed in Pellis et al. 2014; Pellis and Pellis 2017). For example, rats deprived of social interaction during adolescence displayed reduced social interaction with a peer (van den Berg et al. 1999; Lukkes et al. 2009), awkward behaviour with a sexual partner in adulthood (van den Berg et al. 1999), greater aggressive behaviour with peers (Wall et al. 2012), abnormal behaviour in situations of conflict (Einon and Potegal 1991; van den Berg et al. 1999; Tulogdi et al. 2014), and impaired social recognition (Shahar-Gold et al. 2013). Brain regions involved in initiating and regulating social behaviour such as the amygdala, lateral septum, prefrontal cortex, and hippocampus, undergo changes in volume, dendritic spine density, white matter, neuronal density, and neurogenesis from adolescence to adulthood (Mizukami et al. 1983; Verney et al. 1987; Markham et al. 2007; He and Crews 2007; Koss et al. 2014; Willing and Juraska 2015). Thus, social experience may influence ongoing brain development in adolescence. In support of this hypothesis, rats deprived of social interaction had reduced hippocampal volume compared with pair-housed or single-housed rats exposed to a conspecific for 6 min a day (Kalman and Keay 2017), and adolescent single-housed rats had reduced dendritic branching in the orbitofrontal cortex and greater dendritic branching in the medial prefrontal cortex compared

✉ Cheryl M. McCormick
cmccormick@brocku.ca

[1] Department of Psychology, Brock University, St. Catharines, ON L2S 3A1, Canada

[2] Department of Biomedical Sciences, University of Guelph, Guelph, ON N1G 2W1, Canada

[3] Centre for Neuroscience, Brock University, St. Catharines, ON L2S 3A1, Canada

with socially housed adolescents (Bell et al. 2010). Thus, the absence of social behaviour in adolescence alters both social brain and behaviour development.

In turn, the quality of social relationships also influences both social brain and behavioural development. For example, rats that undergo social instability stress in adolescence (SS; daily isolation for 1 h and return to an unfamiliar partner from postnatal days 30–45) show reduced social interactions with unfamiliar peers compared with non-stressed controls (CTL), and this reduction is evident both after the SS procedure in adolescence (Hodges et al. 2017, 2018) and weeks after the procedure in adulthood (Green et al. 2013). The decrease in social interaction is not accompanied by a reduction in the reward value of social interaction; SS and CTL rats do not differ in time spent in social approach to unfamiliar rats separated by wire mesh (Green et al. 2013) or in conditioned place preference for social interaction (Hodges et al. 2017). The pattern of neural activity during social interaction tests (as measured by Fos protein immunoreactivity) across a set of brain regions selected for their involvement in social behaviour (medial amygdala, lateral septum, CA2 subregion of the hippocampus, nucleus accumbens) differed between SS and CTL rats (Hodges et al. 2018). We also investigated the oxytocin system because of its importance in social behaviour (reviewed in Veenema and Neumann 2008), and found increased oxytocin receptor-binding density in the dorsal lateral septum and nucleus accumbens shell in SS rats relative to CTL rats (Hodges et al. 2017). SS and CTL rats differ on a variety of social behaviours other than social interaction; compared with CTL rats, SS rats exhibited increased aggressive behaviour when competing against a peer for a food reward in adulthood (Cumming et al. 2014), showed decreased social recognition (Hodges et al. 2017), and had deficits in mating behaviour with a female (McCormick et al. 2013).

To better elucidate the neural mechanisms that might underlie the differences between SS and CTL rats in social behaviour, here we investigate the effect of adolescent SS on dendritic morphology (number of intersections, dendrite diameter, number and distance of terminals, length of matter, branch order), dendritic spine density, and on markers of synaptic plasticity (spinophilin, synaptophysin, postsynaptic density protein 95 [PSD95], calcium/calmodulin-dependent protein kinase II [CaMKII]) in the medial amygdala and lateral septum. The Golgi–Cox method (e.g., Gibb and Kolb 1998) was used to visualize the dendrites and dendritic spines of neurons in the brain. The medial amygdala and lateral septum were chosen as regions to investigate because of their broad roles in social behaviour. The medial amygdala and lateral septum are involved in social investigation, aggressive behaviour, sexual behaviour, and social recognition (reviewed in Sheehan et al. 2004; Knapska et al. 2007). Markers of synaptic plasticity were chosen based on previous studies that linked them to changes in sociability and social preference (Kim et al. 2013), or because they were found to be altered by exposure to social stressors (Leussis and Andersen 2008; Leussis et al. 2008; Maras et al. 2014). We also investigated glucocorticoid receptor protein expression in the two groups because of the role of glucocorticoids in the structural and functional remodelling of neurons (reviewed in McEwen et al. 2016) and because of the greater exposure to glucocorticoids experienced by SS rats than CTL rats as part of the SS procedure (Hodges and McCormick 2015; McCormick et al. 2007).

# Methods

## Animals

Male Long-Evans rats ($n=40$) were obtained from Charles River, Kingston, New York, on postnatal day (PND) 22 and given a week to acclimate to the animal colony. Rats were housed in pairs and maintained under a 12 h light–dark cycle (lights on at 05:00 h) with food and water available ad libitum. Use of animals in these experiments was approved by the Brock University Institutional Animal Care Committee (ACC) and was carried out in adherence to the Canadian Council on Animal Care guidelines.

## Social instability stress (SS) procedure

Rats were randomly assigned to the adolescent social instability stress (SS, $n=20$) group or to the non-stressed control (CTL, $n=20$) group. The SS procedure was as described previously (reviewed in McCormick 2010; McCormick et al. 2015). Beginning on PND 30, SS rats were isolated in a 12 cm × 10 cm ventilated plastic container in a room separate from the colony for 1 h each day until PND 45. Immediately after isolation each day, SS rats were returned to the animal colony and housed in a new cage with a new cage partner that had also undergone the 1 h isolation. The SS procedure was conducted at various times during the lights on phase of the light–dark cycle to minimize habituation to the procedure. On PND 45, after the final isolation, SS rats remained with the same cage partner and were left undisturbed except for cage maintenance or test procedures. CTL rats remained undisturbed in their home cages except for cage maintenance from time of arrival at the colony until the test procedures.

## Golgi–Cox staining

### Brain collection and Golgi–Cox procedure

A subset of CTL ($n=12$) and SS ($n=12$) rats were decapitated directly upon removal from the home cage on PND 46 and brains were placed in Golgi–Cox impregnation solution for 25 days in the dark. After the 25th day of incubation, brains were placed in sucrose cryoprotectant (30% (w/v) sucrose in phosphate buffered saline, pH 7.4) for 48 h. Brains were then sliced on a vibratome in a 30% sucrose solution into 500 µm-thick sections containing the dorsal lateral septum (from approximately bregma 1.44 mm to bregma − 0.24 mm). Sections were also collected to contain the four subregions of the medial amygdala, which differ based on cytoarchitecture, afferent and efferent projections, and functional differences (Canteras et al. 1995; reviewed in; Newman 1999): the anterodorsal and anteroventral subregions of the medial amygdala (from approximately bregma − 1.92 mm to − 2.64 mm), and the posterodorsal and posteroventral subregions of the medial amygdala (from approximately bregma − 2.76 mm to − 3.48 mm) identified according to Paxinos and Watson (2005). Sections were washed twice in distilled water for 5 min, incubated in 2.7% (v/v) ammonium hydroxide for 15 min, and then washed twice in distilled water for 5 min. Sections were then incubated in ascending steps in ethanol (70%, 70%, 95%, 95% for 8 min each; 100%, 100%, 100% for 5 min each) and cleared in two incubations of xylene for 5 min each. After clearing, sections were mounted onto Superfrost Plus slides (Fisher Scientific, Inc.) and coverslipped using Permount mounting medium (Fisher Scientific, Inc.). During the Golgi–Cox procedure one SS rat brain was damaged and unusable. Thus, the final sample for the Golgi–Cox procedure was $n = 12$ CTL rats and $n = 11$ SS rats.

### Imaging of neurons and analysis of dendrite branches

Imaging and tracing was done blind to experimental condition. Bright field imaging was performed using an upright BX53 microscope (Olympus, Richmond Hill, ON, Canada) fitted with a motorized stage and integrated with Neurolucida software (version 10, MBF Bioscience, Willison, VT). High-resolution z axis image stacks were acquired using an Olympus UPlanSApo 30X, 1.05 NA silicone oil-immersion objective (Olympus, Richmond Hill, ON, Canada; total magnification 300x), and each image stack measured 395 µm (x axis) by 296 µm (y axis) with a z axis step size of 1 µm. For an individual Golgi–Cox-stained neuron of interest, its image stacks overlapped in the x and y axis so that when stitched together in three dimensions the entire neuron was contained. Neurons were randomly sampled from the dorsal part of the dorsal lateral septum, anterodorsal medial amygdala, anteroventral medial amygdala, posterodorsal medial amygdala, and posteroventral medial amygdala, and neurons were equally sampled from both hemispheres. Only stellate neurons were sampled because of their abundance in the medial amygdala (e.g., Dall'Oglio et al. 2008a, b) and lateral septum (e.g., Phelan et al. 1989). A sampled neuron was fully traced and analyzed only if (1) it was fully contained within the section (i.e., no dendrite branches were cut off), (2) it was not occluded by another neuron, and (3) all of its dendrite branches were fully intact. Sholl analyses were used to characterize differences in the dendrite structure of neurons in the medial amygdala and lateral septum between CTL and SS rats. For each rat, three neurons were imaged from each brain region, traced using Neurolucida Software (Version 10, MicroBrightField, Williston, VT, USA), and analyzed using Neurolucida Explorer (MBF Bioscience, Williston, VT, USA). A total of 33–36 dendrites were analysed in each brain region for each experimental group (CTL, $n = 11–12$ rats; SS, $n = 11$ rats). For each neuron, the morphological analysis included the following parameters: (1) the number of intersections of dendritic branching and (2) the dendrite diameter within sholl concentric spheres that radiated outward from the soma in multiples of 25 µm, (3) the number of dendrite terminals, (4) the total amount of dendrite matter in length, (5) the highest branch order of dendrites, and (6) the distance to the most distal dendrite terminal. Values for each rat per brain region were averaged, and these rat averages were used as the level of sampling for statistical analysis. Rats were removed from the statistical analyses for a specific brain region if they did not have three intact neurons in that brain region. See Fig. 1 for examples of the areas from which Golgi–Cox-stained neurons were sampled.

### Dendritic spine analysis

The same neurons traced for dendritic branch analysis were chosen for dendritic spine analysis in all brain regions of interest. Dendrites for dendritic spine analysis had to have clearly visible spines. Three dendrites per brain region per animal were chosen and analysed using Image J and blind to experimental condition. Total spines were counted for the length of 80 µm from the cell body for each dendrite. Spines were counted on a total of 27–33 dendrites in each brain region for each experimental group (CTL, $n = 9–11$; SS, $n = 9–11$). Spine density was calculated as the number of spines per 10 µm length of dendrite and averaged per brain region per rat. Rats were removed from the statistical analyses for a specific brain region if they did not have three dendrites with clearly visible spines.

## Western blotting

### Brain collection, tissue processing, and protein extraction

The remaining CTL ($n = 8$) and SS ($n = 8$) rats were decapitated directly upon removal from the home cage on PND 46, 24 h after the last day of the SS procedure. Brains were extracted, frozen in methylbutane on dry ice, and stored at

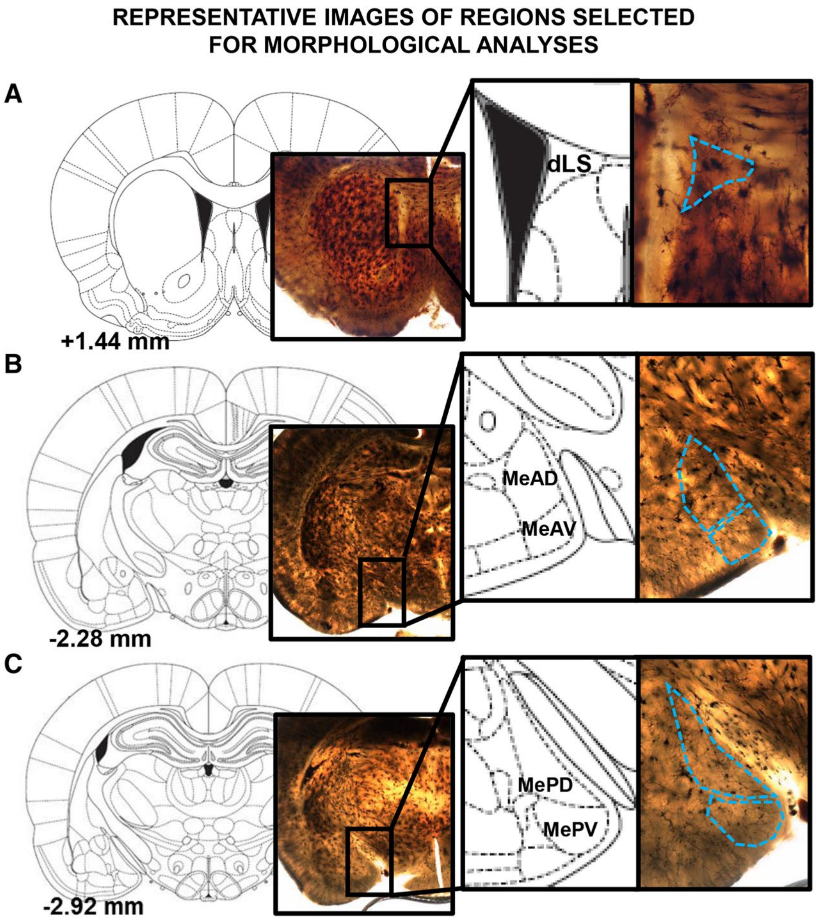

**Fig. 1** Atlas images depicting regions of interest for morphological analyses (images are used with permission from Paxinos and Watson 2005) and corresponding images of Golgi–Cox-stained sections. Brain regions of interest include the dorsal lateral septum (dLS) (**a**), the anterodorsal (MeAD) and anteroventral (MeAV) subregions of the medial amygdala (**b**), and the posterodorsal (MePD) and posteroventral (MePV) subregions of the medial amygdala (**c**). Blue dashed lines represent areas that neurons were samples for morphological analyses

– 80 °C until processing. At the time of processing, brains were sliced into 1.0 mm sections using a chilled brain mould and the slices were placed on glass slides on dry ice. The lateral septum (from approximately bregma 1.44 mm to approximately bregma – 0.24 mm) and the medial amygdala (from approximately bregma – 2.16 to approximately bregma – 3.36 mm) were identified according to Paxinos and Watson (2005), dissected from each hemisphere of each brain using tissue punch tools (1.194 mm inner diameter for lateral septum sections, 0.605 mm inner diameter for medial amygdala sections), and placed into separate microcentrifuge tubes on dry ice. Glass beads (0.5 mm diameter) and ice-cold whole-cell lysis buffer (1 M Tris, 150 mM NaCl, 0.1% Triton-X) with protease and phosphatase inhibitors (Roche, USA) were added into each tube containing tissue. All samples were homogenized using a BulletBlender (NextAdvance, USA) at 4 °C for 3 min at a power setting of 6. After homogenization, samples were incubated on ice for 10 min and then centrifuged at 4 °C at 16 000×*g* for 10 min. After centrifugation, supernatant was collected as whole-cell soluble protein. Protein concentrations were determined using Bio-Rad protein assay (Bio-Rad, USA) and a BioTek Synergy plate reader. Samples were then diluted to equal concentrations using whole-cell lysis buffer, and heated 2× Laemmeli buffer was added to each sample at a 1:1 ratio. Samples were then heated for 5 min at 70 °C, centrifuged at 4 °C at 16 000×*g* for 10 min, and stored at – 20 °C until use in Western blot tests. See Fig. 2 for an example of the areas from which tissue was sampled for western blotting.

### Western blotting

Processed samples were heated for 5 min at 70 °C and 5 µg of protein was loaded onto Bio-Rad TGX stain-free fast-cast 12% polyacrylamide gels (Bio-Rad, USA) for SDS–PAGE. Samples from CTL and SS rats were counterbalanced across gels. Once resolved, gels were illuminated for 5 min under UV light for activation of Bio-Rad stain-free total protein determination. After gel activation, proteins were transferred onto PVDF membranes (EMD Millipore, USA), blocked for 1h in 5% bovine serum albumin in TBS-T (for the primary antibodies anti-spinophilin, anti-synaptophysin, anti-postsynaptic density protein 95 [PSD95], anti-calcium/calmodulin-dependent protein kinase II [CaMKII]) or in 5% milk (for the primary antibody anti-glucocorticoid receptor [GR]) at room temperature, and incubated overnight in primary antibody. Primary antibodies used were anti-spinophilin (1:5000, 06-852 Millipore), anti-synaptophysin (1:5000,

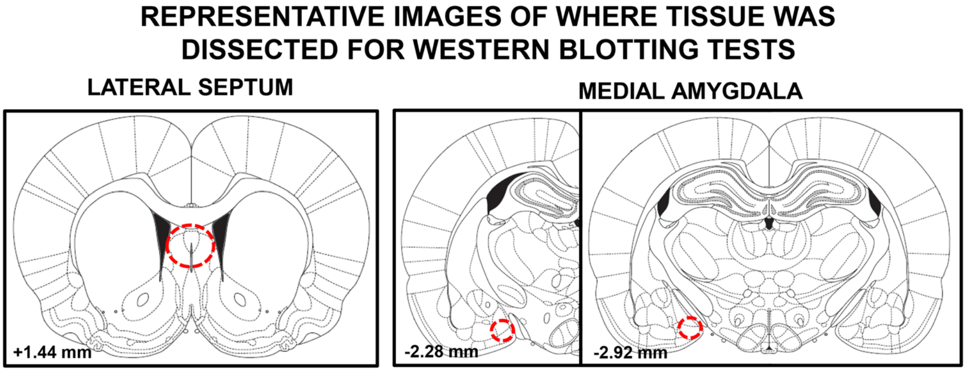

**Fig. 2** Atlas images depicting regions of interest for the western blotting test (images are used with permission from Paxinos and Watson 2005). Red dashed circles represent where tissue was collected for each brain region (tissue collection for the medial amygdala included the anterior and posterior subregions)

MAB5258 Millipore), anti-PSD-95 (1:5000, NB300-556 Novus Biologicals), anti-CaMKII (1:2000, 04-1079 Millipore; detects bands for α and β subunits of CaMKII), and anti-GR (1:200, sc-1004 Santa Cruz Biotechnologies). After incubation in primary antibody, membranes were washed (3 × 5 min washes) in TBS-T, and incubated in secondary antibody (AlexaFluor 488 and AlexaFluor 647; Life Technologies, USA) for 45 min in the dark at room temperature with gentle rocking. After incubation in secondary antibody, membranes were washed in TBS-T, and imaged wet using the Bio-Rad VersaDoc MP4000 (Bio-Rad, USA). Staining intensity (intensity × mm) of each band from each lane was normalized to total lane protein using Bio-Rad stain-free total protein determination (Bio-Rad, USA). Results were divided by a constant for each protein so that they would be represented with the same scale across figures; units are thus arbitrary. During the Western blotting test in the medial amygdala for GR and synaptophysin, parts of the membranes containing one CTL rat and one SS rat were damaged, reducing the sample size to seven per group for these measures.

### Statistics

Statistical analyses were performed using SPSS version 24 software, and consisted of mixed factor analysis of variances (ANOVAs) and independent *t* tests. Post-hoc analyses consisted of *t* tests and Fisher's LSD where appropriate. An alpha level of $p < 0.05$ was used to determine statistical significance in all models. Mixed factor ANOVAs for the three-dimensional Sholl analyses measuring either the average number of intersections or average dendrite diameter consisted of Stress Group (CTL or SS) as the between-groups factor and Distance from Soma (in multiples of 25 µm) as the within-groups factor. Independent *t* tests between SS and CTL rats were performed for the dendritic morphological measures of number of terminals, total dendrite length, highest branch order, and distance to the most distal dendrite terminal. Independent *t* tests between SS and CTL rats were performed for each marker investigated using western blotting.

## Results

### Adolescent social instability increased dendritic arborisation in the anterodorsal subregion and reduced dendritic arborization in the anteroventral and posterodorsal subregions of the medial amygdala

#### Anterodorsal medial amygdala (MeAD)

The interaction between Distance from Soma and Stress Group was significant for the average number of intersections of dendrites in MeAD neurons ($F_{16,320} = 1.770$, $p = 0.034$). The interaction appeared to be driven by a higher average number of intersections of dendrites in the neurons of SS rats than in CTL rat at distances of 25 µm and 350 µm from the soma; nevertheless the post-hoc comparisons were not significant ($p = 0.060$ and $p = 0.067$, respectively; other post-hoc *t* tests, $p > 0.124$). Average dendrite diameter of MeAD neurons decreased based on Distance from Soma ($F_{16,320} = 45.193$, $p < 0.001$), but was not affected by Stress Group ($p = 0.298$). The interaction between Distance from Soma and Stress Group was not significant ($p = 0.596$) (see Fig. 3).

Neurons in the MeAD had an increased number of dendrite terminals ($t_{20} = 2.119$, $p = 0.047$) and an increased highest branch order ($t_{20} = 2.138$, $p = 0.045$) in SS rats compared with CTL rats. The MeAD neurons of CTL and SS rats did not differ in total length of dendrite matter ($p = 0.121$) or in the distance to the most distal dendrite terminal ($p = 0.558$) (see Fig. 3).

#### Anteroventral medial amygdala (MeAV)

The interaction between distance from Soma and Stress Group was significant for the average number of intersections of dendrites in MeAV neurons ($F_{15,300} = 1.842$, $p = 0.029$). The interaction appeared to be driven by a lower average number of intersections in the MeAV neurons of SS rats compared with CTL rats from approximately 50–175 µm

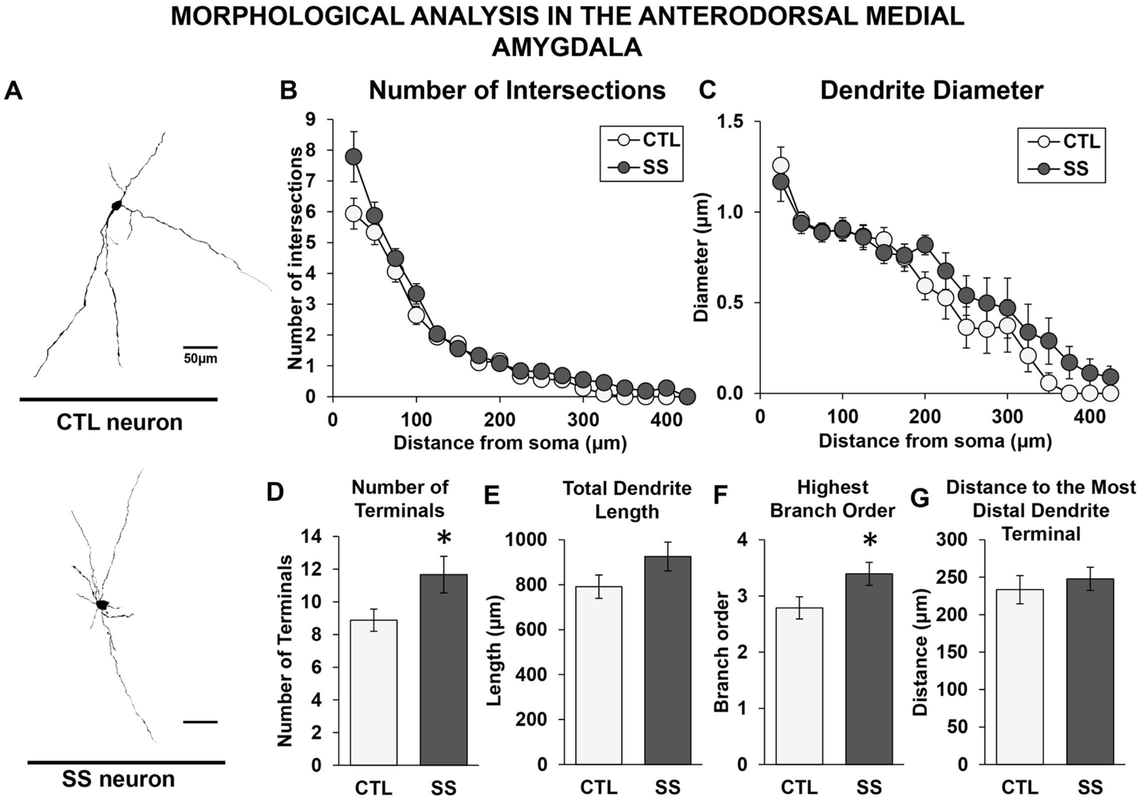

**Fig. 3** Morphological analysis of stellate dendrites in the anterodorsal subregion of the medial amygdala in rats that underwent adolescent social instability (SS) and non-stressed controls (CTL). Representative z-projections of traced neurons are shown in (**a**) for CTL and SS rats. Three-dimensional Sholl analyses measuring the number of dendritic intersections (**b**) and the average dendrite diameter (**c**) within concentric spheres radiating outward from the soma in 25 μm increments, the total number of dendrite terminals (**d**), the total length of dendrite matter (**e**), the highest branch order (**f**), and the distance to the most distal dendrite (**g**) for CTL and SS rats. Data are shown as rat averages (± S. E. M.). **b** The three-dimensional Sholl analysis measuring the number of dendritic intersections revealed a significant interaction of Distance from Soma and Stress Group ($p < 0.05$). **c** Average dendrite diameter decreased based on Distance from Soma ($p < 0.001$). *Indicates a significant increase in SS rats compared with CTL rats in (**d**) the total number of terminals ($p < 0.05$) and **f** the highest branch order ($p < 0.05$)

from the soma. Post-hoc t-tests found a lower average number of intersections in the MeAV neurons of SS rats compared with CTL rats at 50 μm ($t_{20} = 2.250$, $p = 0.036$), 75 μm ($t_{20} = 2.432$, $p = 0.025$), and 100 μm ($t_{20} = 2.311$, $p = 0.032$) from the soma. The lower average number of intersections of SS rats compared with CTL rats at the distances of 150 μm ($p = 0.070$) and 175 μm ($p = 0.071$) did not meet statistical significance (other post-hoc *t* tests, $p > 0.133$). Average dendrite diameter of MeAV neurons decreased based on Distance from Soma ($F_{15,300} = 29.667$, $p < 0.001$), but was not affected by Stress Group ($p = 0.827$). The interaction between Distance from Soma and Stress Group was not significant ($p = 0.995$) (see Fig. 4).

Neurons in the MeAV had a reduced total length of dendrite matter in SS rats compared with CTL rats ($t_{20} = 2.400$, $p = 0.026$). The MeAV neurons of CTL and SS rats did not differ in the total number of dendrite terminals ($p = 0.556$), highest branch order ($p = 0.851$), or in the distance to the most distal dendrite terminal ($p = 0.157$) (see Fig. 4).

### Posterodorsal medial amygdala (MePD)

The average number of intersections of dendrites in MePD neurons decreased based on distance from Soma ($F_{21,441} = 145.765$, $p < 0.001$), but was not affected by Stress Group ($p = 0.301$). The interaction between distance from Soma and Stress Group was not significant ($p = 0.973$). Average dendrite diameter of MePD neurons decreased based on Distance from Soma ($F_{21,441} = 57.297$, $p < 0.001$), but was not affected by Stress Group ($p = 0.508$). The interaction between distance from Soma and Stress Group was not significant ($p = 0.918$) (see Fig. 5).

Neurons in the MePD had a reduced number of dendrite terminals in SS rats compared with CTL rats ($t_{21} = 2.355$, $p = 0.028$). The MePD neurons of CTL and SS rats did not differ in total length of dendrite matter ($p = 0.382$), in highest branch order ($p = 0.139$), or in the distance to the most distal dendrite terminal ($p = 0.867$) (see Fig. 5).

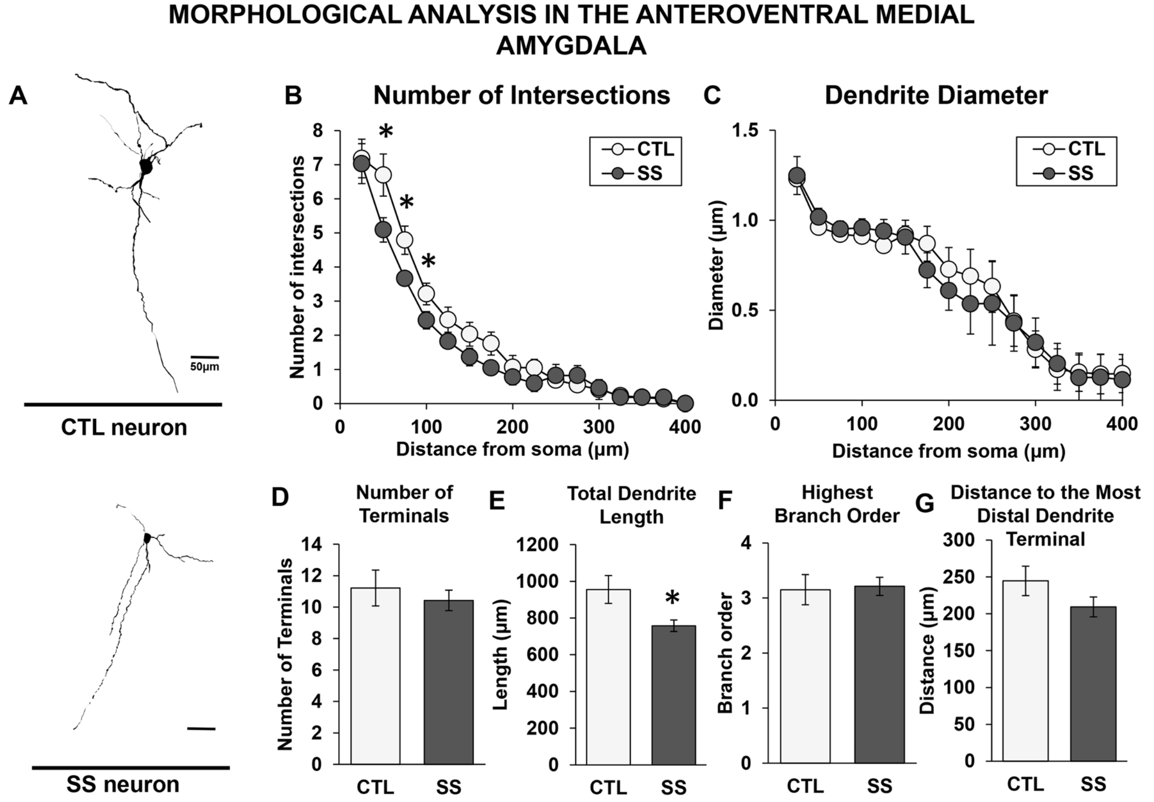

**Fig. 4** Morphological analysis of stellate dendrites in the anteroventral subregion of the medial amygdala in rats that underwent adolescent social instability (SS) and non-stressed controls (CTL). Representative z-projections of traced neurons are shown in (**a**) for CTL and SS rats. Three-dimensional Sholl analyses measuring the number of dendritic intersections (**b**) and the average dendrite diameter (**c**) within concentric spheres radiating outward from the soma in 25 µm increments, the total number of dendrite terminals (**d**), the total length of dendrite matter (**e**), the highest branch order (**f**), and the distance to the most distal dendrite (**g**) for CTL and SS rats. Data are shown as rat averages (± S. E. M.). **b** The three-dimensional Sholl analysis measuring the number of dendritic intersections revealed a significant interaction of distance from Soma and Stress Group ($p < 0.05$). **c** Average dendrite diameter decreased based on Distance from Soma ($p < 0.001$). *Indicates a significant reduction in SS rats compared with CTL rats in **b** the number of intersections ($p < 0.05$) and **e** in the total length of dendrite matter ($p < 0.05$)

### Posteroventral medial amygdala (MePV)

The average number of intersections of dendrites in MePV neurons decreased based on Distance from Soma ($F_{22,462} = 84.088$, $p < 0.001$), but was not affected by Stress Group ($p = 0.591$). The interaction between distance from Soma and Stress Group was not significant ($p = 0.788$). Average dendrite diameter of MePV neurons decreased based on Distance from Soma ($F_{22,462} = 37.613$, $p < 0.001$), but was not affected by Stress Group ($p = 0.590$). The interaction between Distance from Soma and Stress Group was not significant ($p = 0.909$) (see Fig. 6).

The MePV neurons of CTL and SS rats did not differ in the total number of dendrite terminals ($p = 0.431$), total length of dendrite matter ($p = 0.867$), in highest branch order ($p = 0.676$), or in the distance to the most distal dendrite terminal ($p = 0.850$) (see Fig. 6).

### Dorsal lateral septum (dLS)

The average number of intersections of dendrites in dLS neurons decreased based on Distance from Soma ($F_{13,273} = 132.178$, $p < 0.001$), but was not affected by Stress Group ($p = 0.897$). The interaction between Distance from Soma and Stress Group was not significant ($p = 0.999$). Average dendrite diameter of dLS neurons decreased based on distance from Soma ($F_{13,273} = 102.596$, $p < 0.001$), but was not affected by Stress Group ($p = 0.788$). The interaction between distance from Soma and Stress Group was not significant ($p = 0.575$) (see Fig. 7).

The dLS neurons of CTL and SS rats did not differ in the total number of dendrite terminals ($p = 0.557$), total length of dendrite matter ($p = 0.957$), in highest branch order ($p = 0.747$), or in the distance to the most distal dendrite terminal ($p = 0.835$) (see Fig. 7).

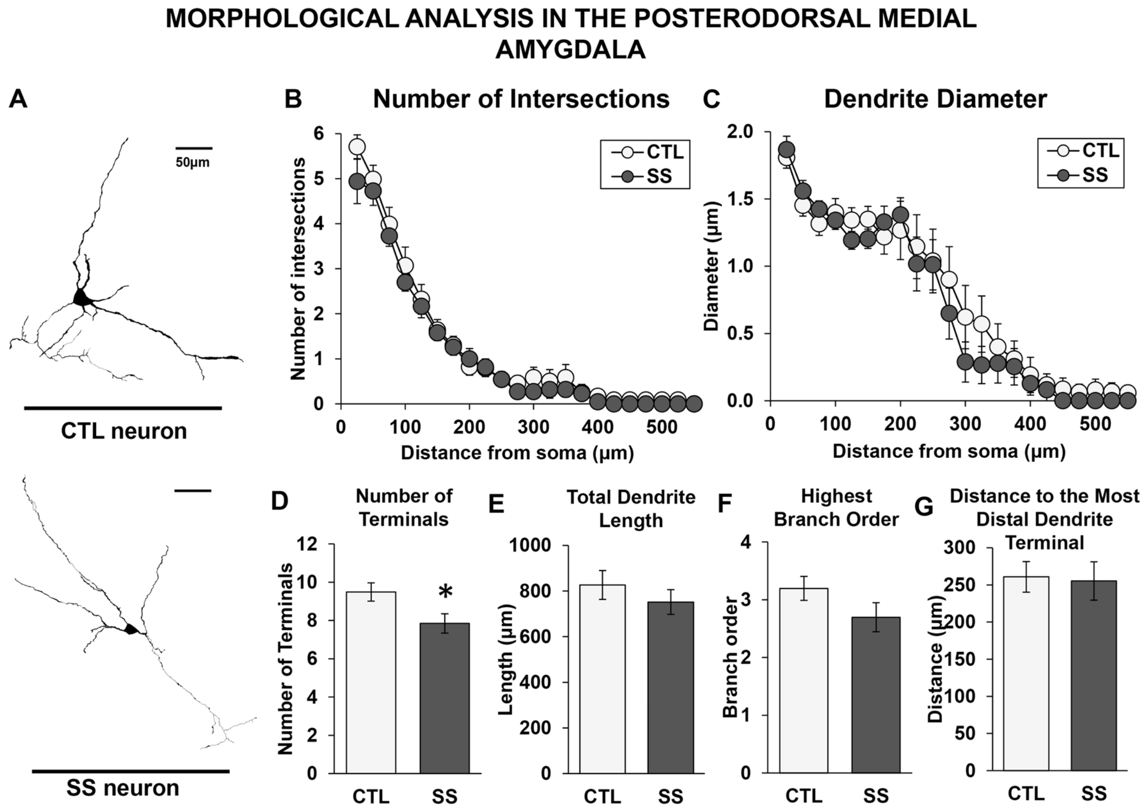

**Fig. 5** Morphological analysis of stellate dendrites in the posterodorsal subregion of the medial amygdala in rats that underwent adolescent social instability (SS) and non-stressed controls (CTL). Representative z-projections of traced neurons are shown in (**a**) for CTL and SS rats. Three-dimensional Sholl analyses measuring the number of dendritic intersections (**b**) and the average dendrite diameter (**c**) within concentric spheres radiating outward from the soma in 25 µm increments, the total number of dendrite terminals (**d**), the total length of dendrite matter (**e**), the highest branch order (**f**), and the distance to the most distal dendrite (**g**) for CTL and SS rats. Data are shown as rat averages (±S. E. M.). **b** The number of dendritic intersections and **c** the average dendrite diameter both decreased based on distance from Soma ($p$'s $< 0.001$). *Indicates a significant reduction in SS rats compared with CTL rats in **d** the total number of dendrite terminals ($p < 0.05$)

### Adolescent social instability reduced dendritic spine density in the dorsal lateral septum

SS rats had lower spine density per 10 µm of dendrites in the dLS than did CTL rats ($t_{18} = 2.181$, $p = 0.043$). CTL and SS rats did not differ in spine density in any subregion of the medial amygdala (all $p > 0.463$) (see Fig. 8).

### Adolescent social instability reduced synaptophysin in the medial amygdala and increased CaMKII in the lateral septum

In the medial amygdala, SS rats had lower synaptophysin protein expression than did CTL rats ($t_{12} = 2.464$, $p = 0.03$), and protein expression for all other markers of interest did not differ between CTL and SS rats (all $p > 0.357$). In the lateral septum, SS rats had higher CaMKII (α and β subunits combined) protein expression than did CTL rats ($t_{14} = 2.680$, $p = 0.018$), and protein expression for all other markers of interest did not differ between CTL and SS rats (all $p > 0.506$) (see Fig. 9).

## Discussion

We tested the hypothesis that the dendritic morphology and synaptic plasticity of the medial amygdala and the lateral septum, which undergo structural development during adolescence (De Vries et al. 1981; Mizukami et al. 1983; Hines et al. 1992; Verney et al. 1987), are susceptible to remodelling by social instability stress (SS) in adolescence. We found that SS in adolescence altered dendritic structure in the medial amygdala, with the direction of difference between SS and non-stressed controls (CTL) dependent on the subregion of the medial amygdala, and reduced dendritic spine density in the dorsal lateral septum compared with CTL rats. SS rats also had reduced synaptophysin in the medial amygdala and increased calcium/

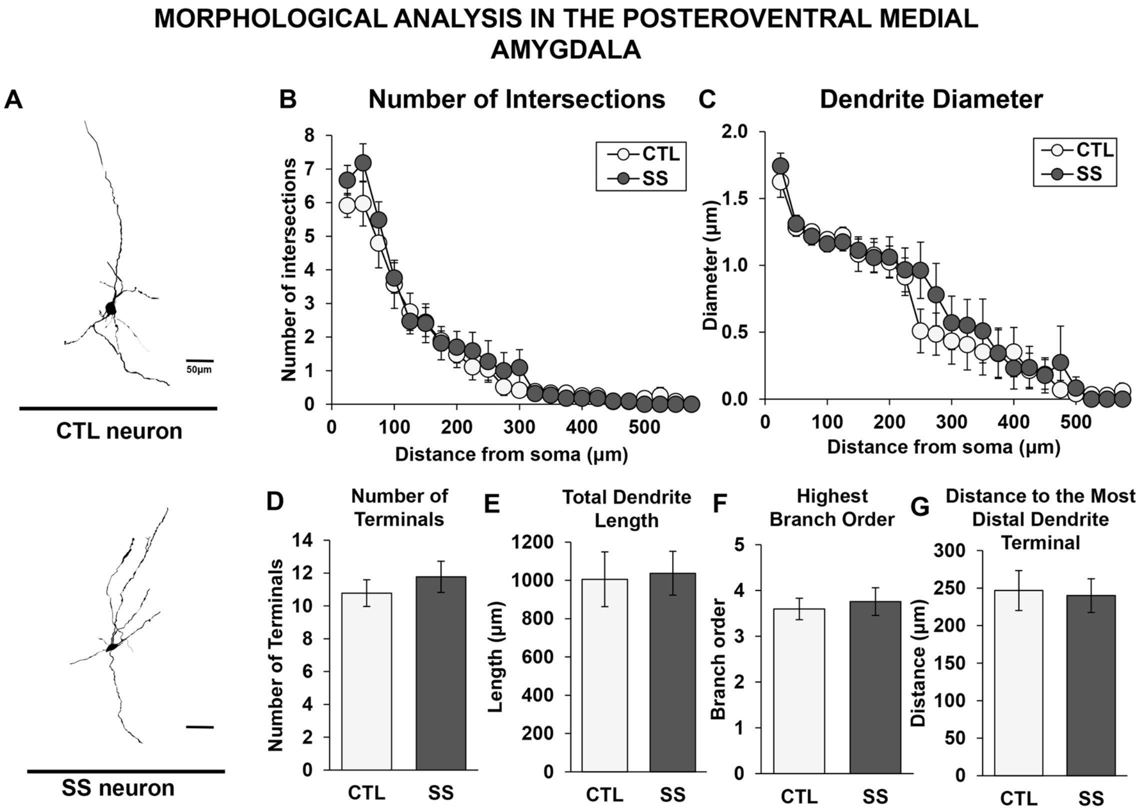

**Fig. 6** Morphological analysis of stellate dendrites in the posteroventral subregion of the medial amygdala in rats that underwent adolescent social instability (SS) and non-stressed controls (CTL). Representative z-projections of traced neurons are shown in (**a**) for CTL and SS rats. Three-dimensional Sholl analyses measuring the number of dendritic intersections (**b**) and the average dendrite diameter (**c**) within concentric spheres radiating outward from the Soma in 25 μm increments, the total number of dendrite terminals (**d**), the total length of dendrite matter (**e**), the highest branch order (**f**), and the distance to the most distal dendrite (**g**) for CTL and SS rats. Data are shown as rat averages (±S. E. M.). **b** The number of dendritic intersections and **c** the average dendrite diameter both decreased based on distance from Soma (*p*'s < 0.001)

calmodulin-dependent protein kinase II (CaMKII) in the lateral septum compared with CTL rats, but did not differ in spinophilin, postsynaptic density protein 95 (PSD95), or glucocorticoid receptor (GR) protein expression in the medial amygdala or lateral septum. The basis for the differences in dendritic complexity between SS and CTL rats likely involves the elevated exposure to glucocorticoids experienced as part of the SS procedure (McCormick et al. 2007; Hodges and McCormick 2015), as glucocorticoids are known to influence the dendritic arbor (reviewed in McEwen et al. 2016).

## Dendritic morphology in the medial amygdala

Adolescent SS increased the number of dendritic intersections in the anterodorsal subregion of the medial amygdala (MeAD) and reduced the number of dendritic intersections in the anteroventral subregion of the medial amygdala (MeAV) in regions close to the soma compared with CTL rats. SS rats also had an increased number of dendrite terminals and higher average branch order in the MeAD and reduced total length of dendrite matter in the MeAV. In the posterodorsal subregion of the medial amygdala (MePD), SS rats had a reduced number of dendritic terminals compared with CTL rats. No differences in dendritic arborisation were observed in the posteroventral subregion of the medial amygdala (MePV) and no differences in dendrite diameter were found in any subregion between SS and CTL rats. That the direction of differences in dendritic complexity differed across regions is consistent with previous reports. For example, glucocorticoid-induced increases in spine densities in the basolateral amygdala were accompanied by increased anxiety, whereas decreases in spine densities in the medial prefrontal cortex were accompanied by impaired working memory (Kim et al. 2014). Glucocorticoid receptor densities are greater in the ventral than dorsal regions of the medial amygdala (reviewed in Herman et al. 2005), and whereas the MePD is critically important for the male social behaviour, the MeAD has been proposed to modulate the display of general arousal rather

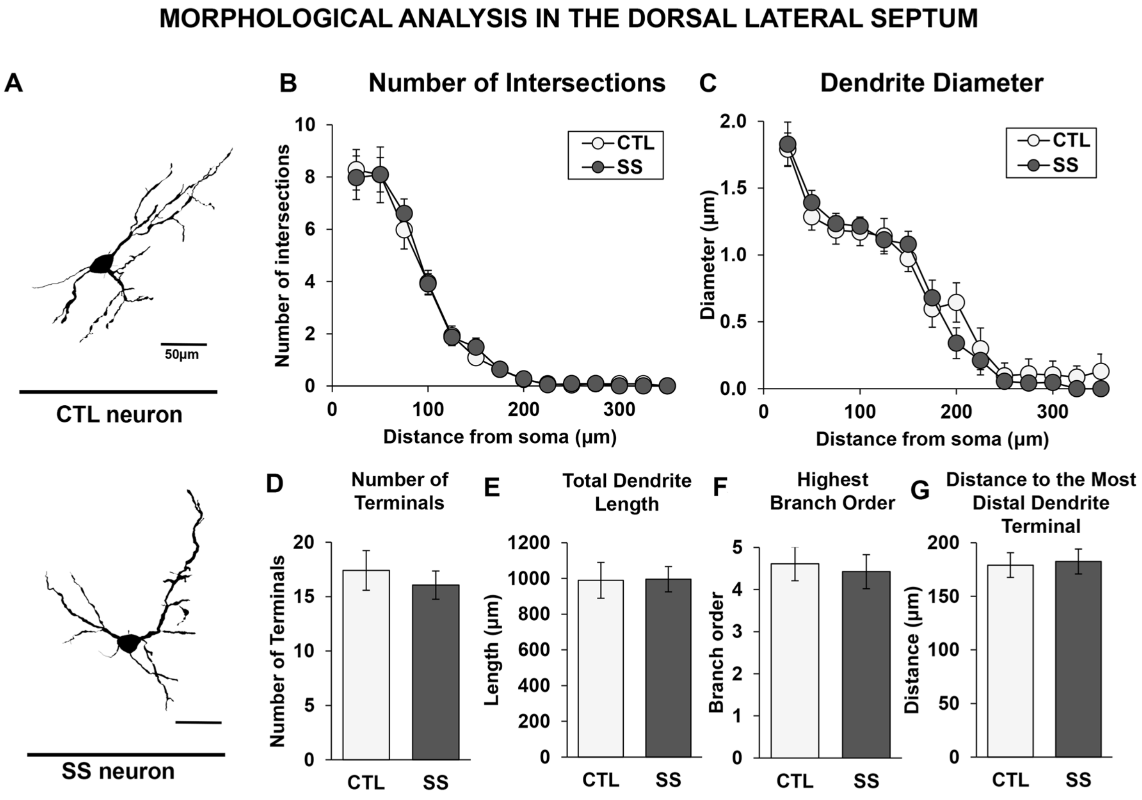

**Fig. 7** Morphological analysis of stellate dendrites in the dorsal lateral septum in rats that underwent adolescent social instability (SS) and non-stressed controls (CTL). Representative z-projections of traced neurons are shown in (**a**) for CTL and SS rats. Three-dimensional Sholl analyses measuring the number of dendritic intersections (**b**) and the average dendrite diameter (**c**) within concentric spheres radiating outward from the soma in 25 µm increments, the total number of dendrite terminals (**d**), the total length of dendrite matter (**e**), the highest branch order (**f**), and the distance to the most distal dendrite (**g**) for CTL and SS rats. Data are shown as rat averages (± S. E. M.). **b** The number of dendritic intersections and **c** the average dendrite diameter both decreased based on distance from Soma ($p$'s $< 0.001$)

than social behaviour, specifically (Newman 1999). Thus, the reduced complexity of dendritic structure in the MePD may underlie the deficits observed in social behaviour in our previous investigations in SS rats (Hodges and McCormick 2019). The reduced complexity of dendritic structure in the MePD in SS rats is consistent with our finding of reduced neural activity (as measured by immediate early gene expression) in the MePD after the 16th isolation and new cage partner pairings relative to age-matched rats undergoing a first isolation and pairing with a new cage partner (Hodges and McCormick 2015). Decreases in neural activity are associated with reductions in dendritic complexity (e.g., Leite-Morris et al. 2014). Thus, when the reduction in synaptophysin in SS rats (described in a later section) also is considered, the evidence suggests the reduced complexity of dendritic structure results in fewer synapses and therefore less neural activity in SS rats than in CTL rats. Together, these results also are consistent with our hypothesis that the repeated elevation in glucocorticoids that result from the repeated isolation and pairing with new cage partners is the basis for the changes in the medial amygdala.

No other study has investigated how social stressors in adolescent rats influence dendritic morphology in the medial amygdala. One study in mice, however, found reduced length of dendritic matter and number of intersections in medial amygdala stellate neurons after daily 2 h restraint in late adolescence into adulthood (PND 49 to 70) (Lau et al. 2017). Others have reported effects of adolescent stressors in other amygdalar nuclei. For example, a reduced number of dendritic intersections in the basolateral amygdala within the range of 20 µm to 90 µm from the soma was found in male rats that underwent a different form of social instability stress in adolescence (1 h immobilization stress and return to different (2 of 5) cagemates daily from PND 28 to 63) compared with non-stressed controls (Tsai et al. 2014). Daily 20 min restraint from PND 25 to 31 increased the total length of dendrite matter in the lateral amygdala (Padival et al. 2015) and exposure to repeated restraint for 6 h daily from PND 20 to 41 increased total length of dendrite matter

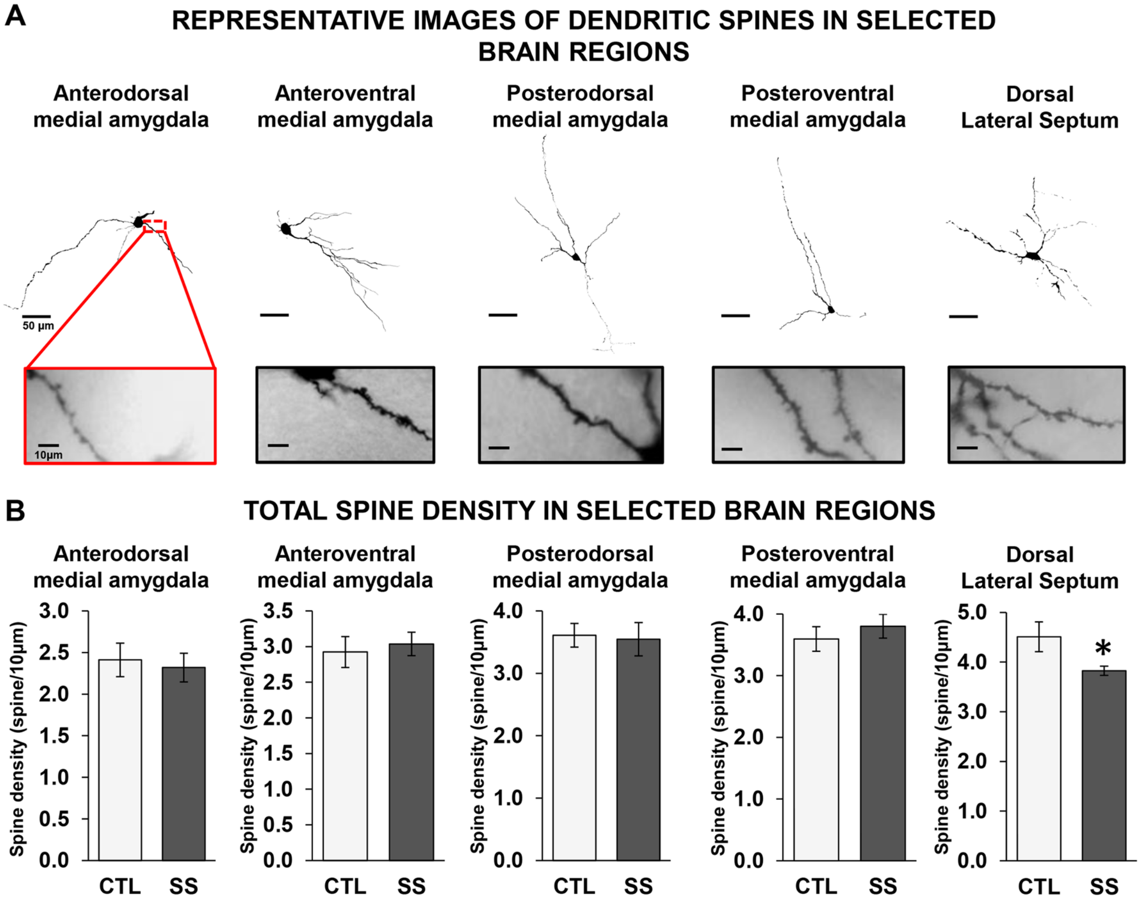

**Fig. 8** Representative z-projections of traced neurons and images depicting dendrites (**a**) and mean (± S. E. M.) total spine density (**b**) in the anterodorsal medial amygdala, the anteroventral medial amygdala, the posterodorsal medial amygdala, the posteroventral medial amygdala, and in the dorsal lateral septum of rats that underwent adolescent social instability (SS) and non-stressed controls (CTL). *Indicates an effect of Stress Group ($p < 0.05$)

in the basolateral amygdala (Eiland et al. 2012) compared with controls. Our lack of findings of changes in dendritic spine density in the medial amygdala of SS rats is in contrast to previous findings of reduced dendritic spine density in the MePD after acute stressor exposure in adult rats (Marcuzzo et al. 2007) and in the total medial amygdala after repeated stressor exposure in adult mice (Bennur et al. 2007). Changes in dendritic spine density in the medial amygdala between CTL and SS rats may have been observed at higher branch orders or at farther distances away from the soma. Nevertheless, changes previously observed in spine density in the medial amygdala likely depend on the subregion, the type of stressor, and on the age range during which the stressor is administered.

Our focus on the medial amygdala is because of its importance in social function (reviewed in Knapska et al. 2007). All subregions of the medial amygdala receive fibres from the main and accessory olfactory bulbs and the vomeronasal organ (Pro-Sistiaga et al. 2007; Mohedano-Moriano et al. 2007) and are involved in processing social stimuli from opposite-sex and same-sex conspecifics (Meredith and Westberry 2004; Kang et al. 2009). Nevertheless, the subregions of the medial amygdala send distinct efferent projections to other parts of the social brain to influence social behaviours (Canteras et al. 1995) and differ in how they process social stimuli. For example, both male and female odours increase Fos immunoreactivity in the MePD and only female odours increase Fos immunoreactivity in the MeAD in male rats (Donato Jr. et al. 2010). Sex hormones in the medial amygdala during puberty are necessary for the expression of adult male behaviour (Sano et al. 2016; reviewed in; Sisk et al. 2016) and blocking sex hormone receptors in the MePD (the subregion with the highest density of androgen and estrogen receptors; Scordalakes et al. 2002), but not in other subregions, influences the social preferences of both males and females (Hosokawa and Chiba 2010; Fujiwara et al. 2016).

Reduced dendritic branching in the MeAV and MePD may play be a basis for the reduced time spent in social interaction with unfamiliar male peers previously found in SS rats compared with CTL rats on PND 46 (Hodges et al.

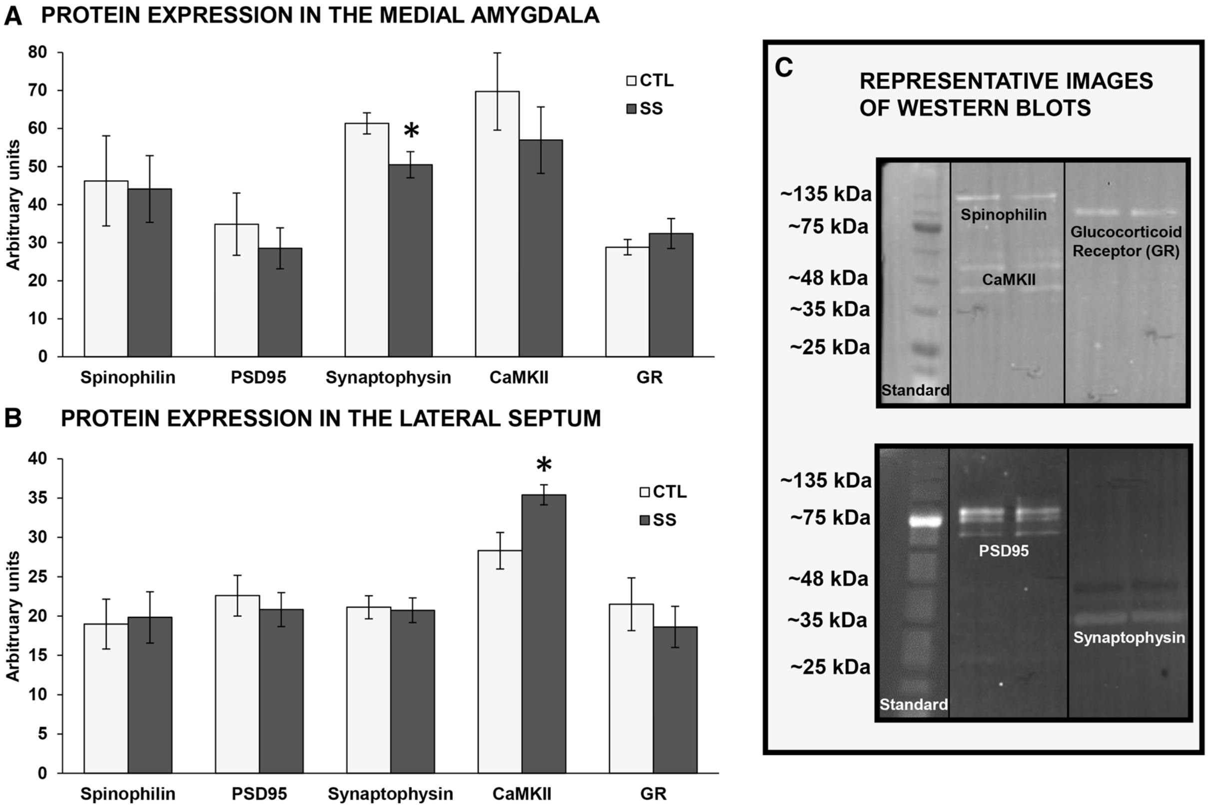

**Fig. 9** Mean (± S. E. M.) protein expression in the medial amygdala (**a**) and in the lateral septum (**b**) of rats that underwent adolescent social instability (SS) and non-stressed controls (CTL), and representative western blot bands for each marker of interest and standard protein ladders (**c**). *Indicates an effect of Stress Group ($p < 0.05$)

2017, 2018). Projections from the accessory olfactory bulb (found to undergo synaptic change in response to male–male social interactions, Cansler et al. 2017) are found in deep cell layers of the MeAV (Mohedano-Moriano et al. 2007), and the MePD responded to male odours (Donato Jr. et al. 2010) and influenced time spent investigating male conspecifics (Adekunbi et al. 2018) in male rats. The MeAD has greater involvement in the olfactory investigation of female conspecifics compared to male conspecifics in male rats (Donato Jr. et al. 2010), and the MePD also plays a role in social interactions with the opposite sex (Adekunbi et al. 2018). The increased dendritic branching in the MeAD and reduced dendritic branching in the MePD on PND 46 suggest that social interactions with females may also be altered in adolescent SS compared with CTL male rats, but this has yet to be tested. Axons of the MeAD and MePV are heavily interconnected (Canteras et al. 1995), yet less is known about the function of the MePV. Our findings suggest that neurons in the MePV may not play a role in the altered social behaviour observed in adolescent SS rats.

Adolescent SS rats spent less time interacting with same-sex unfamiliar peers than did CTL rats on PND 46 (Hodges et al. 2017, 2018) and PND 70 (Green et al. 2013). Thus, the structural changes induced by adolescent social stress in the social brain may also be lasting. Moreover, other behaviours that involve the MeAD and MePD, such as sexual behaviour (Bialy et al. 2002; Dass and Vyas 2014; Hosokawa and Chiba 2005) and aggressive behaviour (Veening et al. 2005; Unger et al. 2015), are both altered in adolescent SS rats tested in adulthood; As adults, SS rats had impaired reproductive behaviour compared with that of CTL rats (McCormick et al. 2013), and females were able to discriminate between SS and CTL adult males (McCormick et al. 2013, 2017). As adults, SS rats displayed greater aggression against their cage partner when competing for access to sweetened condensed milk (Cumming et al. 2014; McCormick et al. 2013, 2017). Future studies measuring dendritic morphology in the social brain regions in adolescent SS rats weeks after the procedure are thus warranted. In addition, female rats that undergo adolescent SS are exposed to higher amounts of corticosterone after the procedure compared with females that were repeatedly isolated and returned to their cagemate daily (McCormick et al. 2007) and we have recently found that adolescent SS female rats also have reduced social interaction with unfamiliar peers compared with CTL females when tested soon after the procedure in adolescence or in adulthood (Asgari et al. unpublished data). Thus, investigation of the social brain regions of female rats after adolescent SS are warranted.

## Dendritic morphology in the dorsal lateral septum

After either adult or adolescent stressor exposures, reduced dendritic spine density in the hippocampus and prefrontal cortex and increased spine density in the amygdala are typically found (reviewed in Radley and Morrison 2005). For example, exposure to social defeat in adolescent mice (PND 35 to 45) reduced the number of stubby spines in the CA1 part of the hippocampus compared with non-stressed controls (Iniguez et al. 2016) and 9 days of repeated restraint in adult rats increased spine density in the basal amygdala (Padival et al. 2013). In contrast, daily 20 min restraint from PND 25 to 31 reduced dendritic spine density in the lateral amygdala in male rats (Padival et al. 2015). To our knowledge, this is the first report of stressors in adolescence reducing spine numbers in the lateral septum. The lateral septum is involved in the exploration of novel stimuli (reviewed in O'Connell and Hofmann 2011), and the neuropeptide oxytocin in the lateral septum has been found to regulate time spent in social interaction (Veenema et al. 2012; Bredewold et al. 2014), the social recognition of familiar and novel peers (Lukas et al. 2013), and social anxiety (Guzmán et al. 2013; Zoicas et al. 2014), behaviours for which SS rats differ from CTL rats (Green et al. 2013; Hodges et al. 2017, 2018). Further, SS rats had increased oxytocin receptor binding in the dorsal lateral septum compared with CTL rats (Hodges et al. 2017). Reduced spine density in the lateral septum of SS rats compared with CTL rats may play a role in our previous findings of reduced social interaction with unfamiliar peers and impaired social recognition in SS rats compared with CTL rats on PND 46 (Hodges et al. 2017, 2018).

## Markers of synaptic plasticity

The proteins synaptophysin, spinophilin, PSD95, and CaMKII were measured in the medial amygdala and lateral septum. Synaptophysin is found in the presynaptic small vesicles of neurons and in neuroendocrine cells, and can be used as an indirect measure of synapse number (Navone et al. 1986; Wiedenmann et al. 1986). Spinophilin (highly enriched in dendritic spines and scaffolding protein) and PSD95 (a major scaffolding protein in the postsynaptic density) have prominent roles in glutamatergic signalling, and also are used as markers of synapse numbers (Allen et al. 1997; reviewed in; Kennedy 1998). CaMKII is an enzyme highly concentrated in the postsynaptic membrane at glutamatergic synapses and is involved in the induction of long-term potentiation (LTP) (reviewed in Fukunaga et al. 1996; Lisman and Zhabotinsky 2001). The only differences found between SS and CTL rats in these proteins was a reduction in synaptophysin in the medial amygdala and an increase in CaMKII in the lateral septum in SS rats relative to CTL rats. Elevated glucocorticoid exposure, such as that found in SS rats compared with CTL rats (McCormick et al. 2007; Hodges and McCormick 2015), results in a brain region-dependent reorganization of synapses (reviewed in Tata and Anderson 2010). For example, stressor/glucocorticoid exposure was found to reduce synaptophysin in the prefrontal cortex, nucleus accumbens, and the hippocampus (Burton et al. 2007; Xu et al. 2004) and increase CaMKII in the basolateral amygdala and the hippocampus (Tran et al. 2011; Zoladz et al. 2012; McCormick et al. 2012). The present findings in concert with our past findings suggest that elevated glucocorticoid exposure in adolescent SS rats may act to reorganize synapses in the medial amygdala and lateral septum.

Others have reported changes in the amount of synaptophysin after social manipulations in adolescence. Adult rats that were isolation-housed from PND 30 to 35 had reduced synaptophysin in the prefrontal cortex (Leussis et al. 2008), and adult rats that were isolation-housed from PND 21 to 28 had reduced synaptophysin in the hippocampus of adult rats (Arcego et al. 2018) compared with socially-housed controls. In those studies, however, changes in synaptophysin were found in adulthood after stressor exposure in adolescence and it remains unknown if the changes we demonstrated here in synaptophysin in adolescent SS rats would still be evident in adulthood; although no differences were found in synaptophysin in the hippocampus between adolescent SS and CTL rats investigated as adults, the medial amygdala was not investigated (McCormick et al. 2012). Reduced synaptophysin in the medial amygdala may reflect the reduced dendritic arborisation we found in SS rats compared with CTL rats in the MeAV and MePD. Gross dissection of the medial amygdala may have obscured any differences in other markers of synaptic plasticity between SS and CTL rats in the medial amygdala; the Golgi–Cox measures indicated that the direction of difference in dendritic morphology varied across subregions of the medial amygdala.

Higher protein expression of CaMKII in the lateral septum of adolescent SS rats compared with CTL rats is in keeping with our past finding of higher protein expression of the alpha subunit of CaMKII in the dorsal hippocampus of SS rats compared with CTL rats when tested in adulthood (McCormick et al. 2012). Glutamatergic signalling was found to regulate the structural plasticity of dendritic spines and enhance dendrite development (Fischer et al. 2000; Mattison et al. 2014). Excess glutamate, however, reduces dendritic growth (Monnerie et al. 2003; Tseng and Firestein 2011). Increased CaMKII, and thus an increase in glutamatergic signalling, in the lateral septum may play a role in reduced spine density in the lateral septum and impaired social behaviour in SS rats compared with CTL rats. In addition to a reduction in social interaction (tests involving physical interaction) with unfamiliar peers in SS rats, we previously reported an increase in time spent in social approach (investigating a peer located behind wire mesh) of unfamiliar peers in SS rats compared with CTL rats (Hodges et al. 2017). Increased CaMKII and increased oxytocin receptor binding in the lateral septum may play a role in the increased social novelty-seeking found in SS rats when rats are behind wire mesh.

SS and CTL rats did not differ in GR protein expression in the medial amygdala and lateral septum. Changes in GR protein expression after repeated stressor exposure have been reported for other brain structures with an increase or decrease dependent on brain region and stressor-type. For example, daily 6 h restraint for 28 days reduced GR protein expression in the prefrontal cortex (Chiba et al. 2012) and daily 2 h immersion in a water bath for 28 days reduced cytosolic GR in the prefrontal cortex and increased nuclear GR in the hippocampus of adult rats (Mizoguchi et al. 2003). Further, 10 days of immobilization stress in adult rats increased GR protein expression in the prelimbic and anterior cingulate cortex compared with non-stressed controls, and these effects were dependent on the activation of the basolateral amygdala (Tripathi et al. 2018). The medial amygdala is responsive to stress exposures (Kellogg et al. 1998; Martinez et al. 1998) and is involved in the activation of the paraventricular nucleus (Dayas et al. 1999) and glucocorticoid release in response to stressor exposure (Solomon et al. 2010; reviewed in; Herman et al. 2005). The SS procedure, although known to elevate glucocorticoid concentrations (McCormick et al. 2007; Hodges and McCormick 2015), may not be sufficient to produce effects on GR densities; in adulthood, adolescent SS rats do not differ from CTL rats in corticosterone release to a number of different stressors (McCormick et al. 2005, 2008; Mathews et al. 2008).

## Conclusion

The interaction of all subregions of the medial amygdala are required to evaluate social odours and influence aggressive, reproductive, and other behaviour output (e.g., in hamsters, Maras and Petrulis 2010a, b; in mice; Cádiz-Moretti et al. 2016), and connections between the lateral septum and medial amygdala are essential for the recognition of familiar and novel individuals (reviewed in Maroun and Wagner 2016). Thus, the structural differences observed in the present experiment in the medial amygdala and lateral septum may underlie the differences observed between SS and CTL rats in a variety of social behaviours (reviewed in Hodges and McCormick 2019). We have postulated that the structural changes in SS rats are the consequence of their elevated exposure to glucocorticoids during the adolescent social stress procedure. In adult rats, many of the stress/glucocorticoid-induced changes in dendritic morphology and behaviour dissipate within days to weeks after cessation of stressors (e.g., Kim et al. 2014). We have proposed that SS alters the ongoing development of social brain structures and thus the effects of SS experienced in adolescence should be evident long after the procedure has ended, which is the case for many behavioural endpoints including social behaviours. It will be important to determine whether the structural changes observed in the present study are long lasting and directly related to the changes in behaviour.

**Acknowledgements** CMM holds a Natural Sciences and Engineering Research Council (NSERC) Discovery Grant that supported the research. TEH holds a NSERC graduate scholarship. The authors have no conflicts of interest to declare.

## References

Adekunbi DA, Li XF, Lass G, Shetty K, Adegoke OA, Yeo SH, Colledge WH, Lightman SL, O'byrne KT (2018). Kisspeptin neurones in the posterodorsal medial amygdala modulate sexual partner preference and anxiety in male mice. J Neuroendocrinol 30 (3):e12572

Allen PB, Ouimet CC, Greengard P (1997) Spinophilin, a novel protein phosphatase 1 binding protein localized to dendritic spines. Proc Natl Acad Sci 94(18):9956–9961

Arcego DM, Toniazzo AP, Krolow R, Lampert C, Berlitz C, dos Santos Garcia E, do Couto Nicola F, Hoppe JB, Gaelzer MM, Klein CP, Lazzaretti C (2018) Impact of high-fat diet and early stress on depressive-like behavior and Hippocampal plasticity in adult male rats. Mol Neurobiol 55(4):2740–2753

Bell HC, Pellis SM, Kolb B (2010) Juvenile peer play experience and the development of the orbitofrontal and medial prefrontal cortices. Behav Brain Res 207(1):7–13

Bennur S, Rao BS, Pawlak R, Strickland S, McEwen BS, Chattarji S (2007) Stress-induced spine loss in the medial amygdala is mediated by tissue-plasminogen activator. Neuroscience 144(1):8–16

Bialy M, Sachs BD (2002) Androgen implants in medial amygdala briefly maintain noncontact erection in castrated male rats. Horm Behav 42(3):345–355

Bredewold R, Smith CJ, Dumais KM, Veenema AH (2014) Sex-specific modulation of juvenile social play behavior by vasopressin and oxytocin depends on social context. Front Behav Neurosci 8:216

Burton CL, Chatterjee D, Chatterjee-Chakraborty M, Lovic V, Grella SL, Steiner M, Fleming AS (2007) Prenatal restraint stress and motherless rearing disrupts expression of plasticity markers and stress-induced corticosterone release in adult female Sprague–Dawley rats. Brain Res 1158:28–38

Cansler HL, Maksimova MA, Meeks JP (2017) Experience-dependent plasticity in accessory olfactory bulb interneurons following male-male social interaction. J Neurosci 28:1031–1037

Canteras NS, Simerly RB, Swanson LW (1995) Organization of projections from the medial nucleus of the amygdala: a PHAL study in the rat. J Comp Neurol 360(2):213–245

Chiba S, Numakawa T, Ninomiya M, Richards MC, Wakabayashi C, Kunugi H (2012) Chronic restraint stress causes anxiety-and depression-like behaviors, downregulates glucocorticoid receptor expression, and attenuates glutamate release induced by brain-derived neurotrophic factor in the prefrontal cortex. Prog Neuro-Psychopharmacol Biol Psychiatry 39(1):112–119

Cumming MJ, Thompson MA, McCormick CM (2014) Adolescent social instability stress increases aggression in a food competition task in adult male Long-Evans rats. Dev Psychobiol 56(7):1575–1588

Cádiz-Moretti B, Otero-García M, Martínez-García F, Lanuza E (2016) Afferent projections to the different medial amygdala subdivisions: a retrograde tracing study in the mouse. Brain Struct Funct 221(2):1033–1065

Dall'Oglio A, Gehlen G, Achaval M, Rasia-Filho AA (2008a) Dendritic branching features of posterodorsal medial amygdala neurons of adult male and female rats: further data based on the Golgi method. Neurosci Lett 430(2):151–156

Dall'Oglio A, Gehlen G, Achaval M, Rasia-Filho AA (2008b) Dendritic branching features of Golgi-impregnated neurons from the "ventral" medial amygdala subnuclei of adult male and female rats. Neurosci Lett 439(3):287–292

Dass SA, Vyas A (2014) Copulation or sensory cues from the female augment Fos expression in arginine vasopressin neurons of the posterodorsal medial amygdala of male rats. Front Zool 11(1):42

Dayas CV, Buller KM, Day TA (1999) Neuroendocrine responses to an emotional stressor: evidence for involvement of the medial but not the central amygdala. Eur J Neurosci 11(7):2312–2322

De Vries GJ, Buds RM, Swaab DF (1981) Ontogeny of the vasopressinergic neurons of the suprachiasmatic nucleus and their extrahypothalamic projections in the rat brain—presence of a sex difference in the lateral septum. Brain Res 218(1–2):67–78

Donato J Jr, Cavalcante JC, Silva RJ, Teixeira AS, Bittencourt JC, Elias CF (2010) Male and female odors induce Fos expression in chemically defined neuronal population. Physiol Behav 99(1):67–77

Eiland L, Ramroop J, Hill MN, Manley J, McEwen BS (2012) Chronic juvenile stress produces corticolimbic dendritic architectural remodeling and modulates emotional behavior in male and female rats. Psychoneuroendocrinology 37(1):39–47

Einon D, Potegal M (1991) Enhanced defense in adult rats deprived of playfighting experience as juveniles. Aggress Behav 17(1):27–40

Fischer M, Kaech S, Wagner U, Brinkhaus H, Matus A (2000) Glutamate receptors regulate actin-based plasticity in dendritic spines. Nat Neurosci 3(9):887–894

Fujiwara M, Nitta A, Chiba A (2016) Regulation of sexual odor preference by sex steroids in the posterodorsal medial amygdala in female rats. Horm Behav 82:46–55

Fukunaga K, Muller D, Miyamoto E (1996) CaM kinase II in long-term potentiation. Neurochem Int 28(4):343–358

Gibb R, Kolb B (1998) A method for vibratome sectioning of Golgi–Cox stained whole rat brain. J Neurosci Methods 79(1):1–4

Green MR, Barnes B, McCormick CM (2013) Social instability stress in adolescence increases anxiety and reduces social interactions in adulthood in male Long–Evans rats. Dev Psychobiol 55(8):849–859

Guzmán YF, Tronson NC, Jovasevic V, Sato K, Guedea AL, Mizukami H, Nishimori K, Radulovic J (2013) Fear-enhancing effects of septal oxytocin receptors. Nat Neurosci 16(9):1185

He J, Crews FT (2007) Neurogenesis decreases during brain maturation from adolescence to adulthood. Pharmacol Biochem Behav 86(2):327–333

Herman JP, Ostrander MM, Mueller NK, Figueiredo H (2005) Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. Prog Neuro-Psychopharmacol Biol Psychiatry 29(8):1201–1213

Hines M, Allen LS, Gorski RA (1992) Sex differences in subregions of the medial nucleus of the amygdala and the bed nucleus of the stria terminalis of the rat. Brain Res 579(2):321–326

Hodges TE, McCormick CM (2015) Adolescent and adult male rats habituate to repeated isolation, but only adolescents sensitize to partner unfamiliarity. Horm Behav 69:16–30

Hodges TE, McCormick CM (2019) Stress and social development in adolescence in a rodent model. Chapter in OC. In: Schultheiss, Mehta P (eds) International handbook of social neuroendocrinology. Taylor & Francis, Routledge, pp 479–503

Hodges TE, Baumbach JL, Marcolin ML, Bredewold R, Veenema AH, McCormick CM (2017) Social instability stress in adolescent male rats reduces social interaction and social recognition performance and increases oxytocin receptor binding. Neuroscience 359:172–182

Hodges TE, Baumbach JL, McCormick CM (2018) Predictors of social instability stress effects on social interaction and anxiety in adolescent male rats. Dev Psychobiol 60(6):1–13

Hosokawa N, Chiba A (2005) Effects of sexual experience on conspecific odor preference and estrous odor-induced activation of the vomeronasal projection pathway and the nucleus accumbens in male rats. Brain Res 1066(1–2):101–108

Hosokawa N, Chiba A (2010) Androgen receptor blockade in the posterodorsal medial amygdala impairs sexual odor preference in male rats. Horm Behav 58(3):493–500

Iñiguez SD, Aubry A, Riggs LM, Alipio JB, Zanca RM, Flores-Ramirez FJ, Hernandez MA, Nieto SJ, Musheyev D, Serrano PA (2016) Social defeat stress induces depression-like behavior and alters spine morphology in the hippocampus of adolescent male C57BL/6 mice. Neurobiol Stress 5:54–64

Kalman E, Keay KA (2017) Hippocampal volume, social interactions, and the expression of the normal repertoire of resident–intruder behavior. Brain Behav 7(9):e00775

Kang N, Baum MJ, Cherry JA (2009) A direct main olfactory bulb projection to the 'vomeronasal'amygdala in female mice selectively responds to volatile pheromones from males. Eur J Neurosci 29(3):624–634

Kellogg CK, Awatramani GB, Piekut DT (1998) Adolescent development alters stressor-induced Fos immunoreactivity in rat brain. Neuroscience 83(3):681–689

Kennedy MB (1998) Signal transduction molecules at the glutamatergic postsynaptic membrane. Brain Res Rev 26(2–3):243–257

Kim KC, Kim P, Go HS, Choi CS, Park JH, Kim HJ, Jeon SJ, dela Pena IC, Han SH, Cheong JH, Ryu JH (2013) Male-specific alteration in excitatory post-synaptic development and social interaction in pre-natal valproic acid exposure model of autism spectrum disorder. J Neurochem 124(6):832–843

Kim H, Yi JH, Choi K, Hong S, Shin KS, Kang SJ (2014) Regional differences in acute corticosterone-induced dendritic remodeling in the rat brain and their behavioral consequences. BMC Neurosci 15(1):65

Knapska E, Radwanska K, Werka T, Kaczmarek L (2007) Functional internal complexity of amygdala: focus on gene activity mapping after behavioral training and drugs of abuse. Physiol Rev 87(4):1113–1173

Koss WA, Belden CE, Hristov AD, Juraska JM (2014) Dendritic remodeling in the adolescent medial prefrontal cortex and the basolateral amygdala of male and female rats. Synapse 68(2):61–72

Lau T, Bigio B, Zelli D, McEwen BS, Nasca C (2017) Stress-induced structural plasticity of medial amygdala stellate neurons and rapid prevention by a candidate antidepressant. Mol Psychiatry 22(2):227

Leite-Morris KA, Kobrin KL, Guy MD, Young AJ, Heinrichs SC, Kaplan GB (2014) Extinction of opiate reward reduces dendritic arborization and c-Fos expression in the nucleus accumbens core. Behav Brain Res 263:51–59

Leussis MP, Andersen SL (2008) Is adolescence a sensitive period for depression? Behavioral and neuroanatomical findings from a social stress model. Synapse 62(1):22–30

Leussis MP, Lawson K, Stone K, Andersen SL (2008) The enduring effects of an adolescent social stressor on synaptic density, part II: Poststress reversal of synaptic loss in the cortex by adinazolam and MK-801. Synapse 62(3):185–192

Lisman JE, Zhabotinsky AM (2001) A model of synaptic memory: a CaMKII/PP1 switch that potentiates transmission by organizing an AMPA receptor anchoring assembly. Neuron 31(2):191–201

Lukas M, Toth I, Veenema AH, Neumann ID (2013) Oxytocin mediates rodent social memory within the lateral septum and the medial amygdala depending on the relevance of the social stimulus: male juvenile versus female adult conspecifics. Psychoneuroendocrinology 38(6):916–926

Lukkes JL, Mokin MV, Scholl JL, Forster GL (2009) Adult rats exposed to early-life social isolation exhibit increased anxiety and conditioned fear behavior, and altered hormonal stress responses. Horm Behav 55(1):248–256

Maras PM, Molet J, Chen Y, Rice C, Ji SG, Solodkin A, Baram TZ (2014) Preferential loss of dorsal-hippocampus synapses underlies memory impairments provoked by short, multimodal stress. Mol Psychiatry 19(7):811

Maras PM, Petrulis A (2010a) Anatomical connections between the anterior and posterodorsal sub-regions of the medial amygdala: integration of odor and hormone signals. Neuroscience 170(2):610–622

Maras PM, Petrulis A (2010b) Lesions that functionally disconnect the anterior and posterodorsal sub-regions of the medial amygdala eliminate opposite-sex odor preference in male Syrian hamsters (Mesocricetus auratus). Neuroscience 165(4):1052–1062

Marcuzzo S, Dall'Oglio A, Ribeiro MF, Achaval M, Rasia-Filho AA (2007) Dendritic spines in the posterodorsal medial amygdala after restraint stress and ageing in rats. Neurosci Lett 424(1):16–21

Markham JA, Morris JR, Juraska JM (2007) Neuron number decreases in the rat ventral, but not dorsal, medial prefrontal cortex between adolescence and adulthood. Neuroscience 144(3):961–968

Maroun M, Wagner S (2016) Oxytocin and memory of emotional stimuli: some dance to remember, some dance to forget. Biol Psychiatry 79(3):203–212

Martinez M, Phillips PJ, Herbert J (1998) Adaptation in patterns of c-fos expression in the brain associated with exposure to either single or repeated social stress in male rats. Eur J Neurosci 10(1):20–33

Mathews IZ, Wilton A, Styles A, McCormick CM (2008) Increased depressive behaviour in females and heightened corticosterone release in males to swim stress after adolescent social stress in rats. Behav Brain Res 190(1):33–40

Mattison HA, Popovkina D, Kao JP, Thompson SM (2014) The role of glutamate in the morphological and physiological development of dendritic spines. Eur J Neurosci 39(11):1761–1770

McCormick CM (2010) An animal model of social instability stress in adolescence and risk for drugs of abuse. Physiol Behav 99(2):194–203

McCormick CM, Cameron NM, Thompson MA, Cumming MJ, Hodges TE, Langett M (2017) The sexual preference of female rats is influenced by males' adolescent social stress history and social status. Horm Behav 89:30–37

McCormick CM, Green MR, Cameron NM, Nixon F, Levy MJ, Clark RA (2013) Deficits in male sexual behavior in adulthood after social instability stress in adolescence in rats. Horm Behav 63(1):5–12

McCormick CM, Hodges TE, Simone JJ (2015) Peer pressures: Social instability stress in adolescence and social deficits in adulthood in a rodent model. Dev Cognit Neurosci 11:2–11

McCormick CM, Merrick A, Secen J, Helmreich DL (2007) Social instability in adolescence alters the central and peripheral hypothalamic-pituitary-adrenal responses to a repeated homotypic stressor in male and female rats. J Neuroendocrinol 19(2):116–126

McCormick CM, Robarts D, Kopeikina K, Kelsey JE (2005) Long-lasting, sex-and age-specific effects of social stressors on corticosterone responses to restraint and on locomotor responses to psychostimulants in rats. Horm Behav 48(1):64–74

McCormick CM, Smith C, Mathews IZ (2008) Effects of chronic social stress in adolescence on anxiety and neuroendocrine response to mild stress in male and female rats. Behav Brain Res 187(2):228–238

McCormick CM, Thomas CM, Sheridan CS, Nixon F, Flynn JA, Mathews IZ (2012) Social instability stress in adolescent male rats alters hippocampal neurogenesis and produces deficits in spatial location memory in adulthood. Hippocampus 22(6):1300–1312

McEwen BS, Nasca C, Gray JD (2016) Stress effects on neuronal structure: hippocampus, amygdala, and prefrontal cortex. Neuropsychopharmacology 41(1):3

Meredith M, Westberry JM (2004) Distinctive responses in the medial amygdala to same-species and different-species pheromones. J Neurosci 24(25):5719–5725

Mizoguchi K, Ishige A, Aburada M, Tabira T (2003) Chronic stress attenuates glucocorticoid negative feedback: involvement of the prefrontal cortex and hippocampus. Neuroscience 119(3):887–897

Mizukami S, Nishizuka M, Arai Y (1983) Sexual difference in nuclear volume and its ontogeny in the rat amygdala. Exp Neurol 79(2):569–575

Mohedano-Moriano A, Pro-Sistiaga P, Úbeda-Bañón I, Crespo C, Insausti R, Martinez-Marcos A (2007) Segregated pathways to the vomeronasal amygdala: differential projections from the anterior and posterior divisions of the accessory olfactory bulb. Eur J Neurosci 25(7):2065–2080

Monnerie H, Shashidhara S, Le Roux PD (2003) Effect of excess extracellular glutamate on dendrite growth from cerebral cortical neurons at 3 days in vitro: Involvement of NMDA receptors. J Neurosci Res 74(5):688–700

Navone F, Jahn R, Di Gioia G, Stukenbrok H, Greengard P, De Camilli P (1986) Protein p38: an integral membrane protein specific for small vesicles of neurons and neuroendocrine cells. J Cell Biol 103(6):2511–2527

Newman SW (1999) The medial extended amygdala in male reproductive behavior a node in the mammalian social behavior network. Ann New York Acad Sci 877(1):242–257

O'connell LA, Hofmann HA (2011) The vertebrate mesolimbic reward system and social behavior network: a comparative synthesis. J Comp Neurol 519(18):3599–3639

Padival MA, Blume SR, Vantrease JE, Rosenkranz JA (2015) Qualitatively different effect of repeated stress during adolescence on principal neuron morphology across lateral and basal nuclei of the rat amygdala. Neuroscience 291:128–145

Padival M, Quinette D, Rosenkranz JA (2013) Effects of repeated stress on excitatory drive of basal amygdala neurons in vivo. Neuropsychopharmacology 38(9):1748

Paxinos G, Watson C (2005) The rat brain in stereotaxic coordinates. Elsevier, Sydney

Pellis SM, Pellis VC (2017) What is play fighting and what is it good for? Learn Behav 45(4):355–366

Pellis SM, Pellis VC, Himmler BT (2014) How play makes for a more adaptable brain: A comparative and neural perspective. Am J Play 7(1):73

Phelan KD, Hasuo H, Twery MJ, Gallagher JP (1989) Projection neurons in the rat dorsolateral septal nucleus possess recurrent axon collaterals. Neurosci Lett 97(3):259–265

Pro-Sistiaga P, Mohedano-Moriano A, Ubeda-Bañon I, del mar Arroyo-Jimenez M, Marcos P, Artacho-Pérula E, Crespo C, Insausti R, Martinez-Marcos A (2007) Convergence of olfactory and vomeronasal projections in the rat basal telencephalon. J Comp Neurol 504(4):346–362

Radley JJ, Morrison JH (2005) Repeated stress and structural plasticity in the brain. Ageing Res Rev 4(2):271–287

Sano K, Nakata M, Musatov S, Morishita M, Sakamoto T, Tsukahara S, Ogawa S (2016) Pubertal activation of estrogen receptor α in the medial amygdala is essential for the full expression of male social behavior in mice. Proc Natl Acad Sci 113(27):7632–7637

Scordalakes EM, Shetty SJ, Rissman EF (2002) Roles of estrogen receptor α and androgen receptor in the regulation of neuronal nitric oxide synthase. J Comp Neurol 453(4):336–344

Shahar-Gold H, Gur R, Wagner S (2013) Rapid and reversible impairments of short-and long-term social recognition memory are caused by acute isolation of adult rats via distinct mechanisms. PloS One 8(5):e65085

Sheehan TP, Chambers RA, Russell DS (2004) Regulation of affect by the lateral septum: implications for neuropsychiatry. Brain Res Rev 46(1):71–117

Sisk CL (2016) Hormone-dependent adolescent organization of socio-sexual behaviors in mammals. Curr Opin Neurobiol 38:63–68

Solomon MB, Jones K, Packard BA, Herman JP (2010) The medial amygdala modulates body weight but not neuroendocrine responses to chronic stress. J Neuroendocrinol 22(1):13–23

Tata DA, Anderson BJ (2010) The effects of chronic glucocorticoid exposure on dendritic length, synapse numbers and glial volume in animal models: implications for hippocampal volume reductions in depression. Physiol Behav 99(2):186–193

Tran TT, Srivareerat M, Alhaider IA, Alkadhi KA (2011) Chronic psychosocial stress enhances long-term depression in a subthreshold amyloid-beta rat model of Alzheimer's disease. J Neurochem 119(2):408–416

Tripathi SJ, Chakraborty S, Srikumar BN, Raju TR, Rao BS (2018) Prevention of chronic immobilization stress-induced enhanced expression of glucocorticoid receptors in the prefrontal cortex by inactivation of basolateral amygdala. J Chem Neuroanat.( **EPub ahead of print**)

Tsai SF, Huang TY, Chang CY, Hsu YC, Chen SJ, Yu L, Kuo YM, Jen CJ (2014) Social instability stress differentially affects amygdalar neuron adaptations and memory performance in adolescent and adult rats. Front Behav Neurosci 8:27

Tseng CY, Firestein BL (2011) The role of PSD-95 and cypin in morphological changes in dendrites following sublethal NMDA exposure. J Neurosci 31(43):15468–15480

Tulogdi Á, Tóth M, Barsvári B, Biró L, Mikics É, Haller J (2014) Effects of resocialization on post-weaning social isolation-induced abnormal aggression and social deficits in rats. Dev Psychobiol 56(1):49–57

Unger EK, Burke KJ, Yang CF, Bender KJ, Fuller PM, Shah NM (2015) Medial amygdalar aromatase neurons regulate aggression in both sexes. Cell Rep 10(4):453–462

van den Berg CL, Hol T, Van Ree JM, Spruijt BM, Everts H, Koolhaas JM (1999) Play is indispensable for an adequate development of coping with social challenges in the rat. Dev Psychobiol 34(2):129–138

Veenema AH, Bredewold R, De Vries GJ (2012) Vasopressin regulates social recognition in juvenile and adult rats of both sexes, but in sex-and age-specific ways. Horm Behav 61(1):50–56

Veenema AH, Neumann ID (2008) Central vasopressin and oxytocin release: regulation of complex social behaviours. Prog Brain Res 170:261–276

Veening JG, Coolen LM, de Jong TR, Joosten HW, de Boer SF, Koolhaas JM, Olivier B (2005) Do similar neural systems subserve aggressive and sexual behaviour in male rats? Insights from c-Fos and pharmacological studies. Eur J Pharmacol 526(1–3):226–239

Verney C, Gaspar P, Alvarez C, Berger B (1987) Postnatal sequential development of dopaminergic and enkephalinergic perineuronal formations in the lateral septal nucleus of the rat correlated with local neuronal maturation. Anat Embryol 176(4):463–475

Wall VL, Fischer EK, Bland ST (2012) Isolation rearing attenuates social interaction-induced expression of immediate early gene protein products in the medial prefrontal cortex of male and female rats. Physiol Behav 107(3):440–450

Wiedenmann B, Franke WW, Kuhn C, Moll R, Gould VE (1986) Synaptophysin: a marker protein for neuroendocrine cells and neoplasms. Proc Natl Acad Sci 83(10):3500–3504

Willing J, Juraska JM (2015) The timing of neuronal loss across adolescence in the medial prefrontal cortex of male and female rats. Neuroscience 301:268–275

Xu H, He J, Richardson JS, Li XM (2004) The response of synaptophysin and microtubule-associated protein 1 to restraint stress in rat hippocampus and its modulation by venlafaxine. J Neurochem 91(6):1380–1388

Zoicas I, Slattery DA, Neumann ID (2014) Brain oxytocin in social fear conditioning and its extinction: involvement of the lateral septum. Neuropsychopharmacology 39:3027–3035

Zoladz PR, Park CR, Halonen JD, Salim S, Alzoubi KH, Srivareerat M, Fleshner M, Alkadhi KA, Diamond DM (2012) Differential expression of molecular markers of synaptic plasticity in the hippocampus, prefrontal cortex, and amygdala in response to spatial learning, predator exposure, and stress-induced amnesia. Hippocampus 22(3):577–589