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**Running title:**Age-related decrease in colonic motility in the mouse

**Key words:** Aging, colonic motility, tachykininsignaling

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**ABSTRACT**

Aging is associated with an increased incidence of constipation in humans. The contribution that the aging process makes to this condition is unclear. The aim of this study was to determine the effects of age on fecal output and colonic motility in male C57BL/6J mice and to determine the role that altered tachykininsignaling plays in this process. Total fecal output recorded over a 24 hour period decreased with age due to a reduction in the number of pellets produced and their water content. These changes occurred in the absence of any significant change in food and water intake. There was an increase in the amount of fecal matter stored in the isolated colon with age which caused a proportional increase in colonic length. Analysis of colonic motility using an artificial pellet demonstrated that pellets moved in a stepwise fashion through the colon. There was an age-related increase in pellet transit time due to decreases in the step distance, velocity, and frequency of stepwise movements. These changes were reversed using the neurokinin 2 ($NK_2$) receptor agonist neurokinin A. Addition of the $NK_2$receptor antagonist GR159897 significantly increased transit time in the young animals by decreasing step distance, velocity and frequency, but was without effect in the aged colon. In summary, the aging C57BL/6J mouse shows animpaired motilityphenotype. These effects appear, at least in part, to be due to an attenuation of tachykininsignaling via $NK_2$ receptors.

**Highlights**

- This study has characterized the effects of age on fecal output, colonic motility and neurokininAsignaling in the mouse.
- Fecal output, pellet water content and colonic motility decreased with age with a corresponding increase in fecal impaction.
- Age-related impairmentsin colonic motility were associated with decreased tachykinin signalling via $NK_2$ receptors

## 1. Introduction

Aging is frequently associated with chronic constipation which can predispose sufferers to fecal impaction and overflow incontinence (Obokhare, 2012). Chronic constipation is a major cause of morbidity in older people and affects around 30-40% of community dwelling adults over the age of 65 years and up to 74% of the institutionalised elderly (Gallagher and O'Mahony, 2009; Rao and Go, 2010). The impact of chronic constipation on quality of life and health care costs is very large (Tariq, 2007). The causes of chronic constipation in the elderly are likely to be multi-factorial and include the effects of age on gastrointestinal (GI) tract physiology, co-morbidities, increased medication use, loss of mobility, reduced caloric intake and ano-rectal sensory changes (Rao and Go, 2010).

Studies using rodents have provided a wealth of information about the physiological mechanisms that regulate GI motility. However, there are limited studies investigating the effects of age on motility. To date only a single study has detailed the changes in fecal output in wild type aged rodents (Smits and Lefebvre, 1996). This study showed a decrease in total fecal output between 12 and 24 month rats, due to a significant reduction in both the number of pellets produced and the wet mass of the pellets. The reasons for these changes are unclear, but the authors suggested that this may include age-related decreases in colonic transit/pellet propulsion and/or reduced tissue excitability, although these were not examined.

In humans, one factor proposed to contribute to age-related constipation is a decrease in the number of myenteric neurons (reviewed in (Camilleri et al., 2008; Saffrey, 2013). Moreover adecrease in cholineacetyltransferasepositive neurons and arelative increase in the percentage of nitric oxide synthase positive neurons have been demonstrated in middle-aged aged adults suffering from slow-transit constipation (Wattchow et al., 2008). However, a reduction in myenteric neuronal number is also seen in humans with no detectable GI disorders and therefore their involvement in age-related constipation is questionable (Bernard et al., 2009). Similarly in animal models the loss of myenteric neurons has also been observed in many of the studies of rodent species although whether the losses are functionally significant is currently unclear (Saffrey, 2013). Reductions in neuronal number have been linked to decreases in migrating motor complex activity in the heterozygous

piebald mouse,although the effects of age in this model were not examined.(Ro et al., 2006).

Irrespective of whether neuronal loss is a determinant of altered function in the aging GI tract, alterations in neuronal signaling processes are likely to play a major role in the direct effect of age on GI motility. Tachykinins are one of theimportant regulators of colonic motility in mice(Brierley et al., 2001; Deiteren et al., 2011;Mulè et al., 2007). Tachykininsregulate colonic motility via $NK_1$ and $NK_2$ receptors. $NK_2$ receptors are predominantly located on longitudinal and circular smooth muscle cellsin both mice (Dickson et al., 2010; Matsumoto et al., 2009) and humans (Giuliani et al., 1991; Jaafari et al., 2007)and provide the main receptor through which electrical field stimulation (EFS)-evoked release of tachykinins causes smooth muscle contraction. In human colons, application of $NK_2$ receptor agonists induced contractions,while selective $NK_2$ antagonists were capable of almost completely blocking EFS-evoked colonic contractions(Giuliani et al., 1991; Nakamura et al., 2011). These data areconsistent with $NK_2$ agonists having a pro-kineticeffect in this part of the GI tract. In addition, both a reduction in the density of tachykininimmunoreactive nerve fibres in human colonic circular muscle (Porter et al., 1998) and decreases in tachykininsignalinghavebeen observed in patients with slow transit constipation consistent with tachykinins having apro-kinetic effect.(King et al., 2010; Stanton et al., 2003).

The contribution that changes in $NK_2$-mediatedsignalingmakes to age-related changes in colonic motility has not previously been examined.This study therefore examined the effects of age on food and water intake, fecal output, artificial pellet propulsion and percentage of the colon full with fecal matter in 3, 12, 18 and 24 month old C57BL/6 mice and the possible role played by altered NKA signalingin these changes.

## 2. Methods

All procedures were carried out according to U.K. Home Office regulations and were approved by the University of Brighton Ethics Committee. Male C57BL/6J mice were obtained from Harlan UK at 8 weeks of age and housed in individual ventilated cages in groups of 3-4, under barrier-reared conditions until required. Animals were maintained at 19.0 ± 1 °C, 55 % humidityand fed on a maintenance diet (irradiated RM1 (E) 801002 chow, Special Diet Services) and had free access to irradiated water.  The animals were kept on a 12 hour light/dark cycle and studied at 3, 12, 18 and 24 months of age.  With the exception of experiments designed to assess 24 hour fecal output, all experiments were carried out between 09.00 and 12.00 hrs.

### 2.1 Assessment of fecal output

Fecal output was analysed by conducting measurements in a metabolic cage for a period of 24 hours on animals from each of the four age groups under the same environmental conditions described above (n=6 per group). For each individual animal, the weight of the food and volume of water consumed during the 24 hour period were calculated. Fecal pellets were removed at the end of the 24 hour period and the total wet weight obtained. Pellet counts were made and the pellets were then left to dry at 50 °C for 24 hours. Total wet and dry fecal output was recorded. For the average fecal pelletweight, the mean weights from 25 dry fecal pellets, sampled at random, were taken from each animal group.

### 2.2 Expulsion of pellets

The entire colon and any fecal pellets it contained was placed in a Sylgard (Corning, UK)-lined organ bath and continuously perfused with oxygenated (95% $O_2$ and 5% $CO_2$) Krebs buffer solution, pH 7.4 (117 mMNaCl, 4.7 mMKCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2$, 1.2 mM $NaH_2PO_4$, 25 mM $NaHCO_3$ and 11 mM glucose) at 37 ± 1 °C at a flow rate of 8 ml min$^{-1}$. The proportion of the colon containingfecal matter was determined in animals of each age group. Images of the colon were taken using Ethovision tracking software (Ethovision XT vs7). The imageswere analysed using Image J, and the ratio of the area of the colon occupied by fecal matter to the total

area of the colon determined. The number of fecal pellets within the colon and the length of the full colon were also recorded. Colon length was also measured following the evacuation of the fecal pellets.

*2.3 Artificial pellet propulsion*

The whole colon was harvested from 3, 12, 18 and 24 month old animals and placed in ice cold Krebs buffer solution. The mesentery was trimmed using fine scissorsand the whole colon was then loosely pinned in a Sylgard-lined flow bath, allowing a lateral movement of approximately 0.5 cm about the mid-line and perfused withoxygenated Krebs buffer solution at 37 ± 1 °C at a flow rate of 8 ml min⁻¹. A small (2 mm) incision was made in both ends of the colon and the openings pinned flat to facilitate pellet insertion and its expulsion at the distal end. If spontaneous evacuation was not achieved, the fecal pellets were removed from the isolated colon after 30 minutes, by gently flushing the lumen of the colon with warmed Krebs buffer solution. The colon was then left to stabilise for 15 minutes, prior to recordings of pellet motility.

Measurements of motility were carried out using an epoxy-coated artificial fecal pellet. A different sized artificial pellet was used for each age group. The choice of fecal pellet utilised foreach of the 4 age groups was based on image analysis of multiple pellets using Image J.20 random pellets from each animal of each age group were analysed for their area, length and width and the average parameters for each age group used toidentifythe fecal pellets used for colonic motility measurements. These 'average' fecal pellets were coated with 3 coats of epoxy prior to *in vitro* monitoring. The artificial fecal pellet was inserted 3-4 mm into the proximal end of the bowel using a fire-polished glass capillary and the movement of the pellet was monitored using a video camera. Pellet motility was tracked using Ethovision tracking software. Following a successful trial, the experiment was repeated two further times and the average response was utilised. Measurements were conducted on all age groups, and the maximum time that a trial was conducted was 45 minutes. The total transit time of the artificial fecal pellet was recorded along with the distance, velocity and frequency of stepwise pellet movements for all age groups. The threshold for a step was defined as the time point when the pellet first moved≥ 2 mm. The end of a step was defined by a period of time ≥10 seconds in which there were no movements of ≥ 2mm.

Measurements were also carried out to study the changes in pellet motility in the presence of the $NK_2$ antagonist, 1 µM GR159897 and the agonist, 1 µM neurokinin A. Briefly, following control recordings, preparations were perfused for a minimum of 20 minutes with either NKA or GR159897 and then a maximum of three pellet motility trials were carried outusing the same epoxy-coated fecal pellet utilised for the control recordings.

### 2.4 Measurements of colonic muscle thickness

Tissues were fixed in 4% paraformaldehyde in phosphate-buffered saline and then embedded in paraffin. Sections were deparaffinised in Histoclear and rehydrated in graded ethanol solutions.Sections were viewed and images were acquired using an Olympus BX-UCB microscope.

### 2.5Statistical methods

Statistical comparison of data was carried out using either a one-way or two-way ANOVA with post hoc Tukey or Bonferroni test and $P < 0.05$ was considered statistically significant. All graphical data was presented as mean ± S.E.M. and $n$ represents the number of animals used for each experiment.

## 3. Results

*3.1 Age-related changes in fecal output*

The average animal weight was 26.4 ± 2.7 g for 3 months, 43.0 ± 5.3 g for 12 months, 41.1 ± 6.3 g for 18 months and 37.5 ± 5.6 g for 24 months old animals (n=20 per group). There was a significant increase in the weight of the animal between 3 months and the other age groups (p<0.001) and between 12 and 24 months (p<0.05). No differences in animal weight were observed between 18 and 24 month old animals.

Animals were placed in the metabolic cage at 09.00hrs. No age-related changes in anxiety were observed as animals of all age groups spent between 5-10 minutes exploring the metabolic cage before spending the rest of the day asleep. Table 1 shows the metabolic cage data from all age groups. There was no significant difference in 24 hour food or water intake with age. The number of fecal pellets decreased between the 3 and 12 month age groups (p<0.001, n=6), with no significant change between the 12, 18 and 24 month groups. Total wet fecal output was significantly reduced in 12 (p<0.01, n=6), 18 (p<0.01, n = 6) and 24 month animals (p<0.001, n = 6) compared to 3 month old animals. Total dry fecal output was significantly decreased in 18 (p<0.05, n=6) and 24 month (p<0.001, n=6) animals compared to 3 month old controls. There was also a significant reduction in dry fecal output in 24 month old animals compared to 12 month old animals (p<0.05, n=6). The average weight of the dry fecal pellet significantly increased between 3 and 18 month old animals (p<0.01, n=6) and significantly decreased between 18 and 24 month old animals (p<0.01, n=6) to a level that was not significantly different from the 3 month group.

Changes in pellet surface area showed a similar pattern to the pellet weight data with a significant increase in pellet surface area between 3 and 18 months (p<0.01) and a decrease in surface area between 18 and 24 months (p<0.001). Water content of the pellets varied between 20-30% for pellets collected from animals between 3 and 18 months and appeared to mimicfluctuations in water intake.

A significant reduction in pellet water content was observed in the 24 month group with values significantly lower than the 3, 12 and 18 month groups (p<0.001, n=6). These changes were not mimicked by a similar decrease in water intake.

*3.2 The proportion of the colon full of pellets and colonic length increases with age.*

Figure 1A shows representative photographic images of isolated whole colons from 3, 12, 18 and 24 month old animals. There was a significant increase in the proportion of the colon full of fecal matter from 3 to 18 months and from 3 to 24 months (p<0.05, n=7 Figure 1B). There was no difference in the fullness of the colon between 18 and 24 month old animals.

The isolated murine colon had an average length of 49.0 ± 3.8 mm for 3 months, 58.4 ± 3.3 mm for 12 months, 62.9 ± 1.1 mm for 18 months and 70.4 ± 1.1 mm for 24 months old animals (n=7) when full (Figure 1C). There was a significant increase in the length of the colon when full in 18 (p<0.01, n=7) and 24 month old animals (p<0.001, n=7) compared to 3 month old animals. There was also a significant increase in the colon length when full in 24 month old animals compared to 12 month old animals (p<0.05, n=7). There was no significant difference in the lengths of the colons when empty with age (n=7, Figure 1D). The number of fecal pellets present in the freshly isolated colon was significantly greater in 18 and 24 month animals compared to 3 month old animals (p<0.001, n=7, Figure 1E). There was also a greater number of fecal pellets in the isolated colon in 18 (p<0.01, n=7) and 24 month animals (p<0.001, n=7, Figure 1E), compared to 12 month animals. No significant difference was observed in the number of pellets observed between 3 and 12 month animals or between 18 and 24 month old animals. Figure 1F shows the percentage reduction of colon length following emptying versus the number of fecal pellets present within the isolated colon. A significant linear relationship was seen suggesting that colon length is a function of the number of pellets held in the colon (p<0.001, n=7).

*3.3 Fecal pellet propulsion decreases with age in the colon*

All preparations of 3, 12 and 18 month old animals were capable of pellet propagation throughout the entire colon within the 45 minute test period. However, only 3 out of 5 24 month colons were able to achieve complete propagation of the artificial fecal pellet.

Figure 2A shows a typical sample trace of pellet motility from each of the four age groups. In all age groups the artificial pellet moved through the colon in a stepwise fashion, with regular waves of contraction transporting the pellet in an aboral direction. The time taken from the insertion of the artificial pellet until its

expulsion from the distal end of the colon was 8.3 ± 1.3mins for 3 months, 10.8 ± 1.7mins for 12 months, 19.3 ± 3.3mins for 18 months and 32.3 ± 4.2mins for 24 months old animals (n=5, Figure 2B). There was a significant increase in time taken for expulsion between 3 month and 24 month old colons (p<0.001, n=5). A similar trend was observed between 12 month and 24 month old colons (p<0.001, n=5). There was also a significant increase in the transit time between 18 and 24 month old colons (p<0.05, n=5). There was no significant difference in pellet transit time between 3, 12 and 18 month old animals.

There was a significant decrease in the distance moved by the artificial fecal pellet during each stepwise movement in 24 month old animals when compared to 3 month old animals (p<0.05, n=5, Figure 2C). The velocity of the artificial pellet during each step significantly decreased in 18 (p<0.01, n=5) and 24 month old animals (p<0.05, n=5) compared to 3 month old animals (Figure 2D). There was also a significant reduction in the frequency of stepwise movements in 18 and 24 month old animals (p<0.05, n=5) compared to 3 month old animals (Figure 2E).

*3.4 The effects of alteringNK$_2$-mediated signaling on pellet motility are age dependent.*

Figure 3 shows the influence of the NK$_2$ antagonist, 1 µM GR159897 and NK$_2$ agonist 1 µM neurokinin A on pellet motility in 3 and 24 month old animals (Figure 3A and B). As described earlier complete pellet transit was seen in all 3 monthold colons but was restricted to 3 out of 5 24 monthold colons.  In the presence of 1 µM GR159897 complete fecal pellettransit was not observed in any of the3and 24 monthold colons tested within the 45 minute test period. (p<0.001, n=6).  Application of 1 µM neurokinin A significantly reduced transittime in 24 month colon (p<0.01, n=6), to rates that were not significantly different to the 3 month group (P>0.05, n=6). In a limited number of experiments these effects of NKA could be completely blocked by 1µM GR159897 suggesting that the effects of NKA were selective for the NK$_2$ receptor (n=3 data not shown).  NKA was without effect in3 month old tissue (p>0.05, n=6).

Further analyses of the individual traces demonstrated thatapplication of the NK$_2$ antagonist GR159897 significantly decreased step distance (Fig 3C), step velocity (Fig. 3D) and the frequency of steps (Fig 3E) in 3 month old colon but was without effect in the 24 month old tissue.  Conversely, application of NKA increased

step distance, step velocity and step frequency in 24 month old colon, to levels that were not significantly different from 3 month controls. Application of NKA was without effect in the 3 month old tissue (Fig 3C, D and E).

## 4. Discussion

### 4.1 Effects of age on fecal output and the properties of fecal pellets

The pharmacological changes that contribute to the effects of age on colonic function have been little studied. In the current study we have shown that fecal output is reduced with age and that this reduction is due primarily to a decrease in the number of pellets produced and a reduction in the water content of the pelletsas the average weight of the fecal pelletsfrom 3 and 24 month old animals was not significantly different.Food intake also remained unchanged with age.  These data are consistent with a similar study performed on rats (Smits and Lefebvre, 1996), but differ from a model of premature aging, the *klotho* mouse, where reduced fecal output was linked to a decrease in food intake (Asuzu et al., 2011).  Increases in the rate of nutrient absorption with age could also contribute to our observations, although this seems unlikely based on earlier studies, which suggest that the rate of absorption of nutrients from the small intestine is reduced with increasing age (Drozdowski and Thomson, 2006; Thomson, 2009).

The water content in the 24 month pellets wassignificantly lower than all the other age groups and was independent of water intake.  A decrease in pellet water content wasalso reported in the rat between 12 and 24 months, although this change reflected a decrease in water consumption (Smits and Lefebvre, 1996).  A decrease in the water content of fecal pellets has also been observed in aged rabbits (Braaten et al., 1988). To prevent disturbance to the animals, pellets were collected at the end of the 24 hour period.  It was therefore possible that changes in the weight of the pellets and their surface area could affect measurements of pellet water content. However, the decrease in water content seen between the 3 and 24 month groups occurred in the absence of any change in pellet surface area/weightand therefore is unlikely to be due to variable rates of evaporation. Variations were seen in the water content of the pellets in the 3, 12 and 18 month groups but these too were independent of water intake.The reduced water content in the 24 month pellets in the absence of any significant change in water intakewould suggest an increase in fluid absorption with age or a decrease in fluid secretion.  Previous work has shown that spontaneous secretory activity is lost in aged guinea pigs and is reduced in response to electrical field stimulation and nicotinic agonists (Powell and Reddix, 2000) and that theophylline stimulated Cl$^-$ secretion is impaired in aged rabbit colon (Braaten et

al., 1988).  This may provide an explanation for the reduced water content seen in our 24 month group.  Additionally, the changes may be reflective of an increase in colonic mucosal permeability with age (Mullin et al., 2002) or the slower transit times seen in the old animals (see below),which would provide extra time for fluid absorption.

### 4.2 Fecal impaction increases with age

Fecal impaction significantly increased with age, and this correlated well with colonic length. In young healthy animals, the length of the colonwas also proportional to fecalcontent(Heredia et al., 2010; Heredia et al., 2012). Similarly, in a  model of partial outlet obstruction that is associated with colonic impaction, colon length was alsoincreased (Heredia et al., 2012).  The relationship between fecal content and colonic length has been termed the 'occult reflex' andis regulated by the activity of a population of nitrergic neurones.  The observations that this reflex exists in aged micesuggests that this reflex pathway is maintained with age.

### 4.3 Effects of age on colonic motility

The effects of age on colonic motility were carried out on isolated whole colons by monitoring the propagation an artificial fecal pellet.Artificial pellets moved in a stepwise manner down the colon as previously described by Brann and Wood (Brann and Wood, 1976).  Transit time was increased with age due to decreases in the frequency and speed of the stepwise movements and the distance moved during each step. A 45% increase in colonic transit time has previously been reported in the aged rat (McDougal et al., 1984), while the rate of propulsion of a solid pellet in isolated Fisher 344 rats was reduced by 20% with age (Wade et al., 2002).  Similar increases in colonic transit times have been recorded in humans with age (Madsen, 1992; Madsen and Graff, 2004; Wiley, 2002), and with age-related constipation (Bhutto and Morley, 2008), consistent with the rodent data, although this change was not seen in all studies (Bernard et al., 2009; Bhutto and Morley, 2008; Hall et al., 2005; Wade and Cowen, 2004).

When investigating if these functional changes could in part bedue to alterations in tachykininsignaling, we observed that application of the $NK_2$ agonist NKA was capable of decreasing colonic transit times in 24 month old colons, but was without effect in 3 month colonsThe lack of effect of NKA on 3 month colon was

unlikely to be because the rate of pellet motility was maximal, becausewe have previously shown that melatonin is capable of increasing the rate of pellet motility in 3 month colon (Diss et al. 2013). This strongly suggests that the lack of effect was because the endogenous levels of tachykinins in the young tissue were maximally stimulating their receptors.Importantly NKA increased step distance, velocity and frequency in 24 month colons to levels that were not significantly different from the young animals. These data suggest that 1) neurokinin signalling was attenuated in the 24 month colon and that 2) the postsynaptic $NK_2$ receptors and their signalling pathways were intact and 3) that the observed changes probably reflected analteration earlier in the tachykininsignalling pathway. Addition of the $NK_2$ antagonist GR159897 decreasedfecal pellet motility in3 month colons but was without effect in the 24 month group consistent with $NK_2$-mediated signaling being naturally impaired in this age group. These results support those from other studies,which have also shown that $NK_2$ blockade can reduce the amplitude of colonic migrating motor complexes (CMMCs), a motility pattern believed to contribute to fecal pellet propulsion(Brierley et al., 2001).The precise causes ofthe decrease in $NK_2$-mediated signaling are unclear and may possibly reflect the loss of myenteric neurones,whichhas been recorded in many species including humans (Saffrey, 2013).However, we have recently shown that myenteric neuronal numbers do not decrease with age in the distal colon of the C57BL/6J male mice suggesting that the changes observed reflect the altered functioning of a fixed neuronal pool (Gamage et al., 2013). It has been suggested that the lack of change in neuronal number in thatstudyis species-specific or reflects subtle changes in the strain of mouse used or the diet that the animals have been maintained on (Kapur, 2013; Saffrey, 2013).

We provide evidence that decreased $NK_2$-mediated signalling may contribute to the reduction in colonic motility observed with age and the impaired motility phenotype, although we cannot exclude the possibility that alterations in other signaling systems are also implicated. Specifically, colonic contractions can also be driven by activity in cholinergic motor neurons, while colonic relaxation, a necessary pre-requisite for aboral pellet motility,is coordinated by a combination of signaling molecules including nitric oxide, a purine and vasoactive intestinal peptide. We have also measureda non-significant increase in the thickness of both the circular and

longitudinal muscle in the distal colon with age, which could also contribute to the observed changes in motility (see Fig. 1 Supplementary data).

*4.4 Conclusions*

In summary, we have characterised the effects of age on fecal output andcolonic motility in the C57BL/6J mouse. Colonic motility is impaired with age and the effects appear at least in part, to be associated with an attenuation of tachykininsignaling via $NK_2$ receptors..

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## Competing interests

None of the authors have any actual or potential conflict of interests.

## Author contributions

NK performed the experiments, analysed the data. SF assisted with experiments and critically evaluated the manuscript. RNR and MJS contributed to the overall project design and critically evaluated the manuscript. BAP assisted with performing the experiments, analysed the data, co-designed the experiments and co-wrote the paper with MSY. The funding and experimental concept was created by MSY.

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**Figure Legends**

**Fig.1.**Fecal matter content of the colon increases with age. (A) Sample photographs taken from freshly isolated colons from 3, 12, 18 and 24 months old animals. (B) Bar graph illustrating the proportion of fecal matter in the colon as a % of colon area. Bar graphs illustrating how colon length changes with age when (C) full of fecal pellets or (D) empty and (E) the effects of age on the number of fecal pellets in the colon. (F) Correlation between the number of fecal pellets in the colon and the % change in colon length following natural emptying of the colon. Values represent mean±SEM, n=12 (parts A, B) or 7 (C-F) for each group. .* $p<0.05$ vs 3 month group.** $p<0.01$, *** $p<0.001$ vs 3 month controls; †† $p<0.05$, ††† $p<0.01$ vs 18 month group; ‡‡ $p<0.01$ vs 12 month group.

**Fig.2.**Colonic motility is decreased with age. (A) Typical sample traces from a 3, 12, 18 and 24 month colon illustrating the movement of an artificial pellet along the colon. The pellet is inserted in the proximal colon and migrates in a stepwise manner to the distal colon where it is expelled. Bar graphs showing the effects of age on mean pellet transit time (B), step distance (C), step velocity (D) and the frequency of steps (E). Values represent mean±SEM, n=5 for each group. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ vs 3 month controls; ††† $p<0.001$ vs 18 month group; ‡ $p<0.05$ vs 12 month group.

**Fig. 3.**NK$_2$-mediated tachykininsignaling is impaired with age. (A) Typical sample traces from a 3 month colon illustrating the movement of an artificial pellet along the colon under control conditions and in the presence of an NK$_2$ agonist (neurokinin A) and an antagonist (GR159897). (B) Similar set of traces for a 24 month colon. Bar graphs showing the effects of age and drug treatment on step distance (C), step velocity (D) and the frequency of steps (E). Values represent mean±SEM, n=6 for each group. *** $p<0.001$ vs 3 month controls; †† $p<0.01$ vs3 month control;‡ $p<0.05$ vs24 month control.

**Table 1.** Effect of age on food intake, water consumption and fecal output. All data obtained from metabolic cage measurements of individual animals of all age groups over a 24 hour period. Data shown as mean ± S.E.M., n=6 where *p<0.05, **p<0.01, ***p<0.001 vs. 3 month old animals; ††p<0.01, †††p<0.001 vs. 12 month old animals and ‡p<0.05, ‡‡p<0.01 and ‡‡‡p<0.001 vs. 18 month old animals

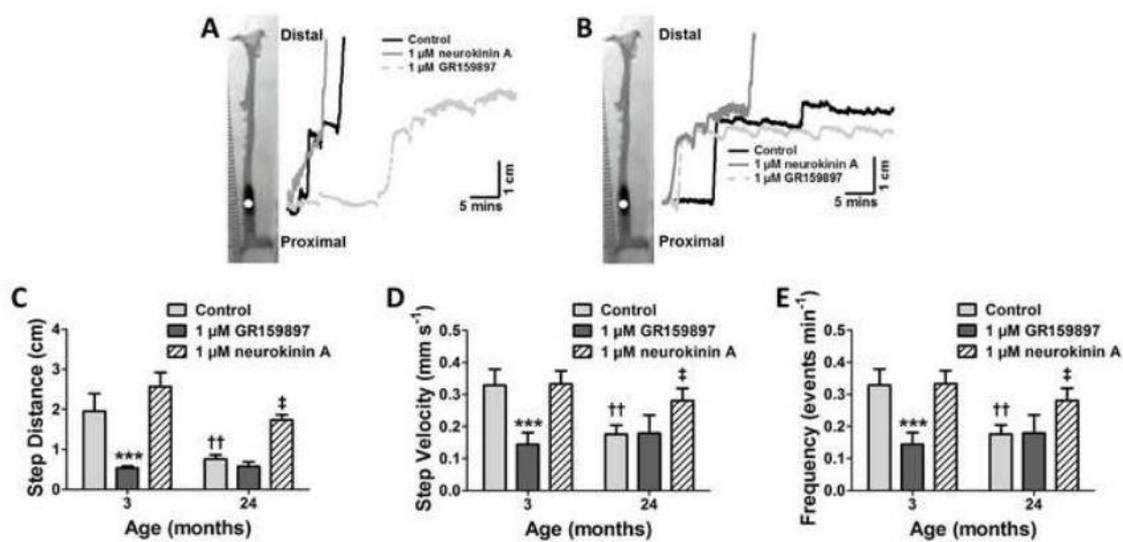| Parameter | Age (months) | | | |
|---|---|---|---|---|
| | 3 | 12 | 18 | 24 |
| Food intake | 4.7 ± 0.5 g | 3.0 ± 0.2 g | 3.5 ± 0.6 g | 2.8 ± 0.5 g |
| Number of fecal pellets | 126 ± 14 | 56 ± 2 *** | 54 ± 6 *** | 45 ± 8 *** |
| Average weight of fecal pellet | 13.9 ± 0.7 mg | 21.6 ± 3.0 mg | 26.9 ± 3.9 mg ** | 12.4 ± 0.7 mg ‡‡ |
| Surface area of fecal pellet | 1.5 ± 0.04 mm$^2$ | 2.0 ± 0.1 mm$^2$ | 2.7 ± 0.3 mm$^2$ ** | 1.2 ± 0.1 mm$^2$ ‡‡‡ |
| Total wet fecal output | 2.0 ± 0.2 g | 1.2 ± 0.1 g ** | 1.2 ± 0.14 g ** | 0.50 ± 0.1 g *** ††‡ |
| Total dry fecal output | 1.3 ± 1.2 g | 0.9 ± 0.04 g | 0.8 ± 0.1 g * | 0.5 ± 0.1 g *** †† |
| Water consumption | 6.2 ± 1.2 ml | 3.3 ± 0.2 ml | 5.3 ± 0.6 ml | 4.2 ± 0.6 ml |
| Water content of fecal pellet | 38.4 ±0.2 % | 21.8 ± 0.2 % *** | 30.8 ± 0.6 % *** ††† | 8.1 ± 1.3 % *** ††† ‡‡‡ |
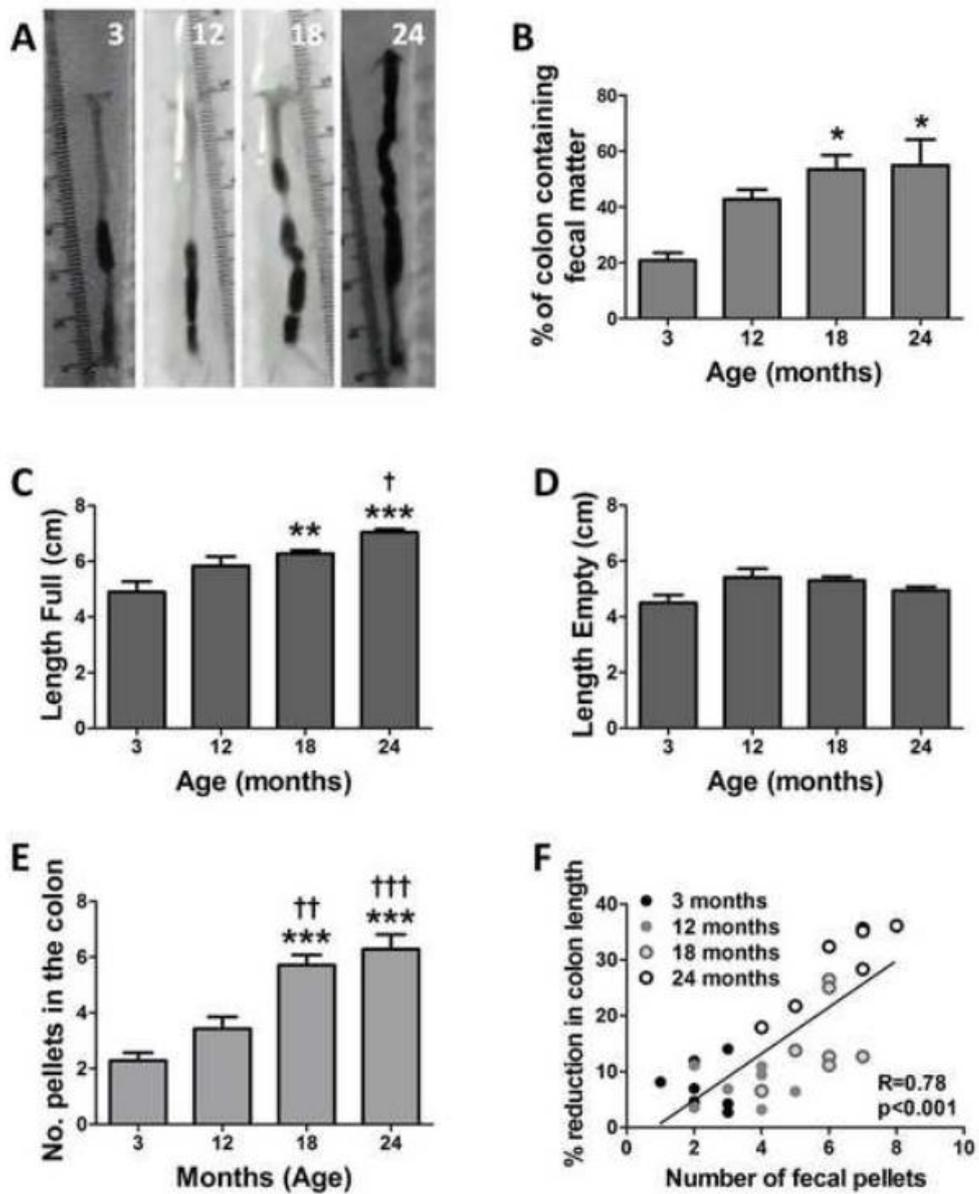
**Fig. 1**
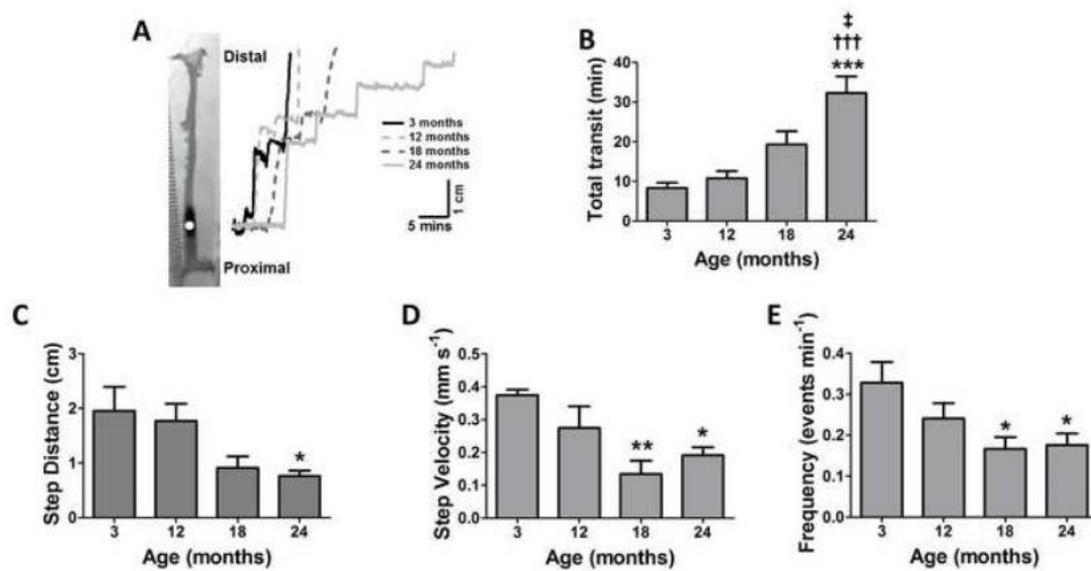
**Fig. 2**

**Fig. 3**

**Highlights**

- This study has characterized the effects of age on fecal output, colonic motility and neurokinin A signaling in the mouse.
- Fecal output, pellet water content and colonic motility decreased with age with a corresponding increase in fecal impaction.
- Age-related impairments in colonic motility were associated with decreased tachykinin signalling via $NK_2$ receptors