A preclinical screen to evaluate pharmacotherapies for the treatment of agitation in dementia

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Agitation associated with dementia is frequently reported clinically but has received little attention in preclinical models of dementia. The current study used a 7PA2 CM intracerebroventricular injection model of Alzheimer's disease (AD) to assess acute memory impairment, and a bilateral intrahippocampal (IH) injection model of AD (aggregated $A\beta_{1-42}$ injections) and a bilateral IH injection model of dementia with Lewy bodies (aggregated NAC_{61-95} injections) to assess chronic memory impairment in the rat. An alternating-lever cyclic-ratio schedule of operant responding was used for data collection, where incorrect lever perseverations measured executive function (memory) and running response rates (RRR) measured behavioral output (agitation). The results indicate that bilateral IH injections of $A\beta_{1-42}$ and bilateral IH injections of NAC_{61-95} decreased memory function and increased RRRs, whereas intracerebroventricular injections of 7PA2 CM decreased memory function but did not increase RRRs. These findings show that using the aggregated peptide IH injection models of dementia to induce chronic neurotoxicity, memory decline was accompanied by elevated behavioral output. This demonstrates that IH peptide injection models of dementia provide a preclinical screen for pharmacological interventions used in the treatment of increased behavioral output (agitation), which also establish detrimental side effects on memory. *Behavioural Pharmacology* 00:000–000 Copyright © 2017 Wolters Kluwer Health, Inc. All rights reserved.

Introduction

A significant number of patients suffering from dementia exhibit behaviors indicative of agitation (Ryu *et al.*, 2005; Savva *et al.*, 2009), such as restlessness and disturbed sleep (Cipriani *et al.*, 2014), and it has been estimated that ~ 90% of dementia sufferers develop behavioral problems that supersede the seminal symptom of memory dysfunction (Tariot and Blazina, 1993). Agitation is found in Alzheimer's disease (AD), dementia with Lewy bodies (DLB), frontotemporal dementia, and other syndromes resulting in dementia (Ballard and Corbett, 2010; Manoochehri and Huey, 2012; Burns and Josephs, 2013). Agitation has been defined as inappropriate verbal, vocal, or motor activity that is unexplained by apparent needs or confusion (Cohen-Mansfield and Billig, 1986). Although agitation might include aggressive behaviors, it can occur without aggression (Cummings *et al.*, 2015), and when it is severe it requires pharmacological treatment (Herrmann and Lanctot, 2007). The treatment of agitation has been identified as an unmet need in relation to adequate care provided for those suffering from cognitive impairments (Herrmann and Lanctot, 2007; Gitlin *et al.*, 2012). When the agitation is severe the symptoms include disinhibition, irritability, aggression, and aberrant motor activity, which affect the patient's quality of life and cause increased stress for the family and caregivers (Kales *et al.*, 2014; Antonsdottir *et al.*, 2015; Cummings *et al.*, 2015; Panza *et al.*, 2015).

This aspect of dementia has been largely overlooked in the employment of laboratory-based animal models of dementia, where the focus has been directed toward establishing neuropathological and behavioral features reflecting a decline in memory abilities. Agitation related to dementia is frequently observed clinically (Lyketsos *et al.*, 2000; Ballard *et al.*, 2001; Cohen-Mansfield, 2013), and various pharmacological interventions have been used to treat this. These treatments include the use of anxiolytic, antidepressant, antipsychotic, and anticonvulsant drugs (Salzman *et al.*, 2008; Kales *et al.*, 2014; Antonsdottir *et al.*, 2015; Cummings *et al.*, 2015; Panza *et al.*, 2015; Soto *et al.*, 2015). The most contentious approach relates to the use of antipsychotic drugs (Jeste *et al.*, 2008), which have been reported to be of only modest value (Schneider *et al.*, 2006a, 2006b; Ballard *et al.*, 2009), and to produce adverse effects (Gitlin *et al.*, 2012). Overall, the currently available pharmacological treatments for agitation in dementia are of little value, and the adverse effects of antipsychotic drugs are of considerable concern (Ballard, 2006; Schneider *et al.*,

2006a, 2006b; Sacchetti et al., 2012; Ma et al., 2014). Basically, there are no officially approved pharmacotherapies for agitation in dementia, and few if any safe and effective pharmacotherapies (Salzman et al., 2008; Kales et al., 2014; Antonsdottir et al., 2015; Cummings et al., 2015; Panza et al., 2015; Soto et al., 2015). Further, nonpharmaceutical approaches have been shown to be of very limited value (Kales et al., 2014; Ballard et al., 2016; Steinberg, 2016). Of the pharmacological treatments used for the management of agitation in dementia, benzodiazepines (anxiolytics) have weak effects (Salzman et al., 2008; Wilson et al., 2012; Kales et al., 2014; Defrancesco et al., 2015; Ngo and Holroyd-Leduc, 2015) and have been found to accelerate cognitive deterioration (Defrancesco et al., 2015), the antidepressants citalopram (Pollock et al., 2002) and sertraline (Lyketsos et al., 2003) have been suggested to have some effects (Sink et al., 2005), but the trial using citalopram had a high dropout rate due to lack of efficacy, and sertraline had no benefit with respect to neuropsychiatric symptoms. Antipsychotics are of modest value (Ballard et al., 2009; Gitlin et al., 2012) but induce adverse cerebrovascular events, especially during the first weeks of treatment (Sacchetti et al., 2012; Wu et al., 2013), and increase mortality (Ballard, 2006; Schneider et al., 2006a, 2006b; Sacchetti et al., 2012; Ma et al., 2014), and anticonvulsants raise concerns with respect to tolerability (Gallagher and Herrmann, 2014). Consequently, a preclinical model of dementia that measures adverse effects on memory (the seminal symptom of dementia), and also models the agitation commonly observed clinically, would be useful for screening pharmacotherapies for the treatment of agitation in dementia.

The most widely known form of dementia is AD, which is associated with the accumulation of amyloid-β (Aβ) plaques in the brain (e.g. Glenner and Wong, 1984). It accounts for up to 80% of all cases of dementia (Hebert et al., 2013) and is comprehensively documented. DLB is the second most common form of dementia: it presents in ∼40% of Parkinson's disease cases (Poewe, 2005), and the severity of DLB correlates significantly with the density of Lewy body deposition in the brain (Hurtig et al., 2000). Whereas $A\beta_{1-42}$ (the most toxic form of Aβ) and $A\beta_{1-40}$ are major components of the aggregated plaques found in AD, aggregated α-synuclein is the major component of Lewy bodies (Spillantini et al., 1998), and the nonamyloid component (NAC) region of α-synuclein, residues 61–95 (NAC_{61-95}), is essential for the aggregation and toxicity of α-synuclein (El-Agnaf et al., 1998). NAC_{61-95} was first isolated from the amyloid plaques associated with AD (Ueda et al., 1993), and this region of α-synuclein has been linked with an increased propensity of α-synuclein to form fibrils (Jethva et al., 2011). The fibrillogenic capacity of NAC_{61-95} is well documented, and the extracellular aggregation of NAC and intracellular accumulation of α-synuclein are considered to be contributory factors in the pathogenesis of DLB and AD.

Consequently, to determine whether an animal model of dementia can reflect the increased behavioral output (agitation) commonly reported in clinical observations of patients suffering from dementia, the current study used a bilateral intrahippocampal (IH) injection model of the effect of aggregated $A\beta_{1-42}$ in AD, and a bilateral IH injection model of the effect of aggregated NAC_{61-95} in DLB to produce chronic neurotoxic peptide deposits in the brain. Although recent theories relating to the onset of dementia, and in particular AD, suggest that the precipitating event is the disruption of synaptic transmission due to the formation of the oligomeric configuration of the neurotoxic peptide (e.g. Haas and Selkoe, 2007; Walsh and Selkoe, 2007; Klyubin et al., 2008; Shankar et al., 2008; Ondrejcak et al., 2010; Pharm et al., 2010), very few patients presenting clinically with symptoms of memory decline are likely to be at such an early stage of disease progression. However, the intracerebroventricular (ICV) 7PA2 CM injection model of AD (e.g. Cleary et al., 2005) was used to determine the effects of acute synaptotoxicity on increased behavioral output, even though clinical management of typical dementia cases generally involves the use of drugs for the treatment of agitation following diagnosis at the stage of pathogenesis when aggregated neurotoxic peptides are chronically established in the brain.

The operant technique employed was the alternating-lever cyclic-ratio (ALCR) schedule (Weldon et al., 1996). This experimental evaluation of behavior has been used to assess the effects of bilateral IH injections of aggregated $A\beta_{1-42}$ (O'Hare et al., 1999; Richardson et al., 2002), bilateral IH injection of aggregated NAC_{61-95} (Kim et al., 2009; O'Hare et al., 2010a, 2010b), and ICV injections of 7PA2 CM in the rat (e.g. Cleary et al., 2005; Poling et al., 2008). The aggregated peptide injections were delivered into the hippocampus as this is an area of the brain directly implicated in learning and memory (Jerrard, 1993), and the ALCR schedule was used as this is recognized as a sensitive, accurate, and parsimonious determinant of memory function in the rat (Cleary et al., 2005; Poling et al., 2008). Also, the ALCR provides data indicating experimental effects on running response rates (RRRs). RRRs illustrate increases or decreases in behavioral output, and increased RRRs may be analogous to the increased behavioral output seen as agitation in human dementia.

The RRR is the rate of lever press responding at any given operant ratio response value, minus the post-reinforcement pause duration (Ferster and Skinner, 1957). The post-reinforcement pause duration is the time spent after receiving a reinforcer (generally a food pellet for experimental animals) before beginning the next

lever press engagement for production of the next reinforcer. Consequently, RRRs provide a measure of actual lever-pressing activity per unit of lever-pressing time. This observation is generally incorporated in operant experiments to ensure that malaise or peripheral motor impedance resulting from an experimental manipulation is not misinterpreted as an adverse central nervous system effect on memory-related behavioral measurements. That is, an observed decline in cognitive (memory) ability that correlates with a reduction in RRRs might simply be a reflection of the physical inability of an animal, due to malaise or illness, to complete a required task (seen as a decrease in activity, and misinterpreted as an effect on memory). In the current study, data on RRRs were collected to determine whether the induction of neurotoxicity might affect RRRs in a manner that indicated the development of agitation. These data were used to determine whether an increase in RRRs, possibly inferring the agitation commonly seen in the syndrome reported for human patients suffering from dementia, was an inherent feature of experimentally induced memory dysfunction due to acute synaptotoxicity (ICV 7PA2 CM injections), or chronic neurotoxicity due to the presence of neurotoxic peptide aggregates in the hippocampal region of the brain.

Methods
Subjects
Forty-eight male Sprague-Dawley rats (Harlan, UK), weighing 229–250 g at the beginning of the experiment, were maintained at 90% of their free-feeding body weights, and housed individually with water freely available in the home cage. The temperature in the vivarium was maintained at 23°C under a 12-h-light/12-h-dark cycle (lights on at 08:00 h). The relative humidity of the vivarium was maintained at 50–65%, and light intensity was controlled at a maximum of 9 lux in the home cage. This work was approved by the relevant institutional ethics committees and conducted under Home Office License (UK).

Apparatus
Ten two-lever rat test chambers (Med Associates Inc., St Albans, New Jersey, USA) enclosed in sound-attenuating compartments were employed. Food reinforcers were 45 mg sucrose food pellets (BioServ, Frenchtown, New Jersey, USA) that were delivered into a tray situated midway between the two operant levers. A Siemens computer programmed in MED-PC (Med Associates Inc.) controlled the experiment and collected data. The operant test chambers had internal dimensions of 30.5 cm length × 24.1 cm width × 21 cm height. The pellet receptacle was 2.9 cm wide × 2.5 cm high × 1.9 cm deep, and was situated in the center of the 24.1 cm chamber wall. The retractable operant response levers were 4.8 cm wide × 1.9 cm deep and were positioned 2.1 cm above the floor of the chamber on each side of the

pellet receptacle. The house light was situated in the center of the chamber ceiling, and when illuminated had an output of 3 W.

Behavioral training
The training procedure adopted has previously been reported extensively (e.g. Weldon *et al.*, 1996; Richardson *et al.*, 2002; Cleary *et al.*, 2005). Briefly, behavioral sessions were conducted 7 days/week, during which the rats were trained to press both levers for food reinforcement, each operant training session lasting for a maximum of 50 min. Approximately 20–30 sessions after the initial lever press training, the ALCR schedule was introduced. Using this schedule, rats alternate to the other lever after pressing the currently correct lever a sufficient number of times to obtain a food reinforcer. The number of lever presses required for each reinforcer changes, increasing from two responses per food pellet to 56 responses per food pellet, and then decreasing back to two responses per food pellet, repeated over six cycles. One complete cycle requires alternating-lever responses of 2, 6, 12, 20, 30, 42, 56, 56, 42, 30, 20, 12, 6, and 2. The requirement to alternate levers generates data on incorrect lever perseverations, which indicate disruption of well-learned behaviors, or reference memory, including general aspects of executive function, reasoning, and goal-oriented manipulation of previously acquired information (Cleary *et al.*, 2005; Poling *et al.*, 2008). The ALCR schedule also measures RRRs; these are response rates at each schedule value, minus the post-reinforcement pause duration. Consequently, RRRs provide a measure of actual lever-pressing activity per unit of lever-pressing time, thereby indicating the general level of activity.

7PA2 conditioned medium
7PA2 cells are stably transfected Chinese hamster ovary cells that incorporate the cDNA for amyloid precursor protein (APP751); this is specific for the familial AD mutation Val171Phe (Podlisny *et al.*, 1998; Shankar *et al.*, 2011). These cells secrete $A\beta_{1-40}$ and $A\beta_{1-42}$ (Shankar *et al.*, 2011), and were grown to just below confluence in Dulbecco's Modified Eagle's medium containing 10% fetal bovine serum and 200 μg/ml G418. They were briefly washed in DPS and incubated at 37°C with 5% CO_2 for 18 h with a sufficient volume of Dulbecco's Modified Eagle's medium to cover the cells. After incubation the medium was centrifuged at 3000*g* for 15 min and snap-frozen and stored at −20°C until thawing for ICV injections. Using enzyme-linked immunosorbent assay, the concentration of total Aβ in the 7PA2 CM was in the range of 2–5 nmol/l.

Aggregation of $A\beta_{1-42}$ and NAC_{61-95}
Aggregated $A\beta_{1-42}$ and aggregated NAC_{61-95} were prepared from solutions of 10^{-4} mol/l soluble $A\beta_{1-42}$ or soluble NAC_{61-95} peptides (Sigma, UK) in 0.01 mol/l filtered PBS (pH 7.4) (Weldon *et al.*, 1998). The $A\beta_{1-42}$

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and NAC_{61-95} solutions were agitated (Teflon-coated stirbar at 200 rpm) at room temperature for 36 h. Following agitation, both solutions ($A\beta_{1-42}$ and NAC_{61-95}) were turbid. The $A\beta_{1-42}$ and NAC_{61-95} peptides were then sedimented by centrifugation (10 min × 15 000g) to 80% sedimentation of each peptide. These sediments of aggregated $A\beta_{1-42}$ and aggregated NAC_{61-95} were then dissolved with PBS, aliquoted, and stored at −20°C before thawing for bilateral IH injections.

Surgical procedure
When all rats were able to complete the ALCR schedule in 50 min without demonstrating changes in operant response trends, they were anesthetized with fentanyl citrate (0.4 ml/kg) and placed in a stereotaxic frame (Kopf, USA). For ICV injections (7PA2 CM administration), 16 rats were fitted with a permanently indwelling cannula (23 G) aimed at the lateral cerebral ventricle. Half of the rats in each randomly assigned group (experimental and control) received left lateral ventricle cannula implants and the other half received right lateral ventricle cannula implants. With the incisor bar set 3.5 mm below the interaural line, the stereotaxic coordinates for cannula implantation were 1.0 mm posterior and ±1.5 mm lateral to Bregma, and 3.0 mm below the pial surface (Paxinos and Watson, 1998). Cannula placement and patency were verified by vigorous drinking (>5 ml/20 min) following ICV injection of 0.5 μg/ml of angiotensin II (Johnson and Epstein, 1974). Following a 7-day recovery period from surgery, the experimental group was injected ICV with 10 μl of 7PA2 CM and the control group was injected ICV with 10 μl of CHO CM (wild-type control), and initial behavioral testing was conducted 2 h following ICV injections. This temporal sequence for behavioral testing following 7PA2 CM ICV injections has been previously explained, and the effect on memory dissipates within 24 h after injection (Cleary et al., 2005).

For IH injections (aggregated $A\beta_{1-42}$ and aggregated NAC_{61-95}) the remaining 32 rats fitted with permanently indwelling bilateral cannulae aimed at the CA3 region of the dorsal hippocampus of the brain, with the incisor bar set 3.5 mm below the interaural line; the stereotaxic coordinates were 3.3 mm posterior and ±2.6 mm lateral to Bregma and 3.7 mm below the pial surface (Paxinos and Watson, 1998). Following a 7-day recovery period from surgery the rats were randomly assigned to groups. Sixteen rats were assigned to the aggregated $A\beta_{1-42}$ experimental group, of which eight received bilateral IH injection of aggregated $A\beta_{1-42}$ and eight received bilateral IH injection of sterile water, and 16 rats were assigned to the aggregated NAC_{61-95} group, of which eight received bilateral IH injection of aggregated NAC_{61-95} and eight received bilateral IH injection of sterile water. These experimental subjects were injected

bilaterally IH with 5 μl (per side) of aggregated $A\beta_{1-42}$ ($n = 8$) and the corresponding control group was injected bilaterally IH with 5 μl (per side) of sterile water ($n = 8$); the remaining IH group was injected bilaterally IH with 5 μl (per side) of aggregated NAC_{61-95} ($n = 8$) and the corresponding control group was injected bilaterally IH with 5 μl (per side) of sterile water ($n = 8$). Using a 26 G needle connected to a Hamilton microsyringe, injectates were deposited slowly over a 15 min period, and the injector remained in place for a further 2 min. Sterile water was used as the control injectate because this was the case in other behavioral studies of this nature, and adhering to this procedure ensured that the data were comparable to those of previously published studies. Ten days after the bilateral IH injections, the collection of behavioral data resumed. Data were collected daily from all six cycles of the ALCR schedule for each subject in each group. Following the collection of behavioral data from the IH-injected groups (aggregated $A\beta_{1-42}$ and aggregated NAC_{61-95} and their IH-injected sterile water control counterparts), these rats were bilaterally injected IH with 1 μl of Evans blue (per side). All of the IH-injected rats were then deeply anesthetized and perfused through the ascending aorta with 4% paraformaldehyde. Using a cryostat, the brains were then sectioned through the plane of the IH microinjection sites and viewed using a light microscope for confirmation of the position of each IH injection.

Statistical analyses
The ICV 7PA2 CM versus CHO CM data on incorrect lever perseverations were analyzed by means of the t-test everyday (Fig. 1), and the ICV 7PA2 CM RRR data were analyzed by repeated-measures analysis of variance (Fig. 2). The aggregated $A\beta_{1-42}$ versus sterile water data and the aggregated NAC_{61-95} versus sterile water data on incorrect lever perseverations were analyzed by t-test (Fig. 3). The RRR data ($A\beta_{1-42}$ and NAC_{61-95}) were analyzed by daily t-tests in the first instance for deviation from the control condition and then by repeated-measures analysis of variance within and between groups (Figs 4 and 5). The two types of data were analyzed differently because the analyses of incorrect lever perseverations tested a-priori predictions about effects at particular timepoints: ICV 7PA2 injection effects on memory have previously been shown to last for less than 24 h, and IH-aggregated peptide injection effects on memory have previously been shown to take ~30 days to develop.

Results
ICV injections of 7PA2 CM produced an acute increase in incorrect lever perseverations (Fig. 1), indicating a decline in cognitive ability ($t_{14} = 5.33$, $P < 0.001$). This acute effect of 7PA2 CM-induced synaptotoxicity on behavior, lasting for less than 1 day, has been previously reported (e.g. Cleary et al., 2005). But ICV 7PA2 CM
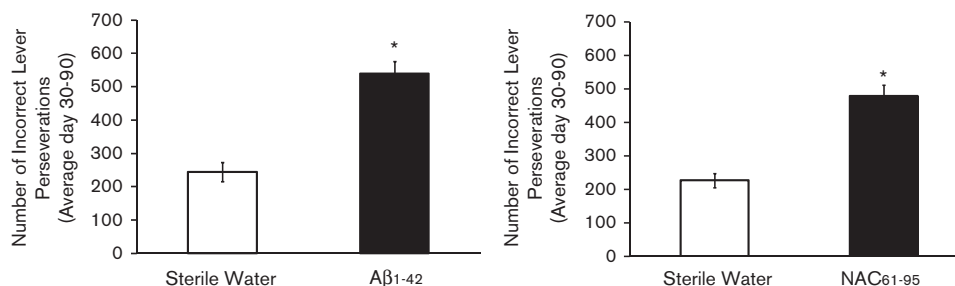
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Fig. 1
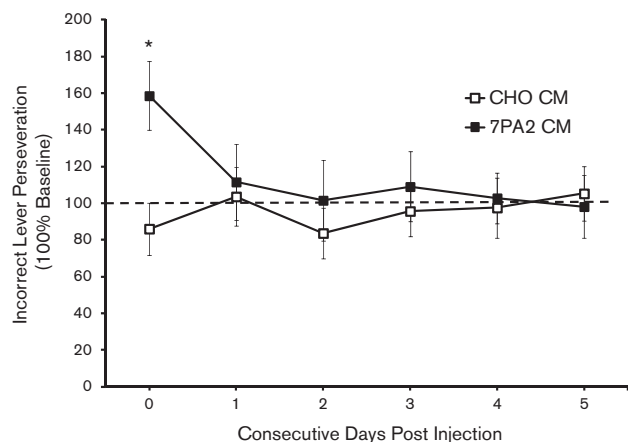
Effect of intracerebroventricular 7PA2 CM injection on incorrect lever perseverations. Data are represented as mean±SEM (*$P < 0.001$).
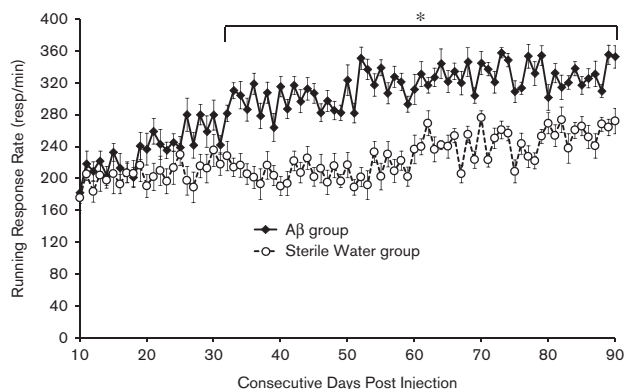
Fig. 2
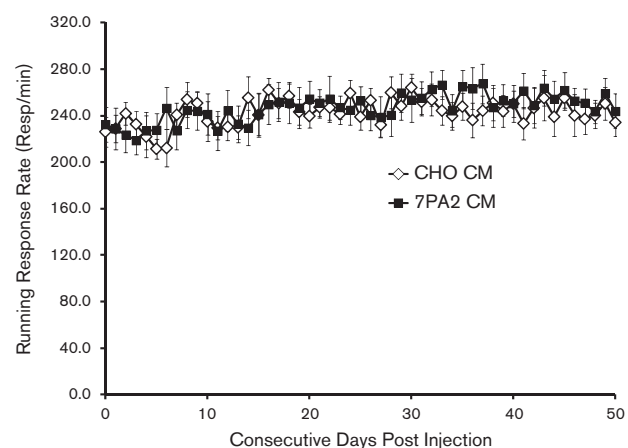
Effect of intracerebroventricular 7PA2 CM injection on running response rates up to 50 days after injection. Data are represented as mean±SEM (repeated-measures analysis of variance within and between groups *P*'s > 0.05).
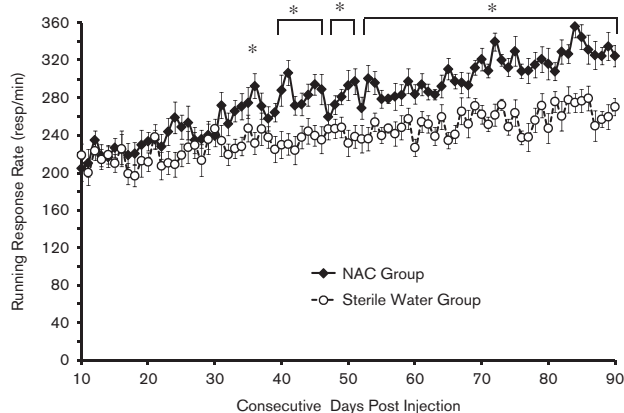
Fig. 4

Effect of bilateral intrahippocampal injections of aggregated Aβ_{1-42} in the CA3 area of the dorsal hippocampus on running response rates. Data are represented as mean±SEM (*$P < 0.001$; repeated-measures analysis of variance within and between groups *P*'s < 0.001). Aβ, amyloid-β.

injection had no acute or chronic (up to 50 days after injection) effect on RRRs within the groups (7PA2 CM: $F_{50,350} = 1.21$, NS; CHO CM: $F_{50,350} = 1.11$, NS) or between the groups ($F_{1,14} = 0.01$, NS) (Fig. 2).

Bilateral IH injection of aggregated Aβ_{1-42}, and bilateral IH injection of aggregated NAC$_{61-95}$ produced a chronic increase in incorrect lever perseverations, indicating a decline in cognitive ability (Aβ_{1-42} vs. sterile water; $t_{14} = -24.62$, $P < 0.001$: NAC$_{61-95}$ vs. sterile water; $t_{14} = -18.89$, $P < 0.001$) (Fig. 3) from approximately day 30 onward. This finding is consistent with the effects of IH-aggregated peptide injections on operant behavior in the rat since its first investigation (Cleary *et al.*, 1995) and has previously been reported for IH injections of aggregated Aβ_{1-42} (e.g. O'Hare *et al.*, 1999) and for aggregated NAC$_{61-95}$ (e.g. Kim *et al.*, 2009) (Aβ_{1-42} vs. sterile water day 1–29; $t = -1.30$, $P > 0.05$: NAC$_{61-95}$ vs. sterile water day 1–29; $t = -1.22$, $P > 0.05$). There were no significant differences in RRRs during approximately the first

Fig. 3

Effect of bilateral intrahippocampal injections of aggregated Aβ_{1-42} and aggregated NAC$_{61-95}$ in the CA3 area of the dorsal hippocampus on incorrect lever perseverations. Data are represented as mean±SEM (*$P < 0.001$). Aβ, amyloid-β; NAC, nonamyloid component.

Fig. 5

Effect of bilateral intrahippocampal injections of aggregated NAC_{61-95} in the CA3 area of the dorsal hippocampus on running response rates. Data are represented as mean ± SEM ($*P < 0.01$; repeated-measures analysis of variance within and between groups $P < 0.001$ and < 0.05, respectively). NAC, nonamyloid component.

35 days after bilateral IH injection of aggregated $A\beta_{1-42}$ (Fig. 4); however, RRRs increased significantly in the aggregated $A\beta_{1-42}$ group from this point until the end of the study (P's < 0.001). Repeated-measures analyses indicated a significant effect within the groups by days ($A\beta_{1-42}$; $F_{80,560} = 8.93$, $P < 0.001$: sterile water; $F_{80,560} = 4.26$, $P < 0.001$) and between the groups ($F_{1,14} = 9.19$, $P < 0.001$). There were no significant differences in RRRs during approximately the first 35 days after bilateral IH-aggregated NAC_{61-95} injection (Fig. 5); however, RRRs increased significantly in the NAC_{61-95} group from this point until the end of the study (P's < 0.01). Repeated-measures analyses indicated a significant effect within the groups by days (NAC_{61-95}; $F_{80,560} = 7.76$, $P < 0.001$: sterile water; $F_{80,560} = 3.82$, $P < 0.001$) and between the groups ($F_{1,14} = 5.51$, $P < 0.05$).

Discussion

The current study employed animal models of the two most prevalent forms of dementia, AD and DLB (Poewe, 2005; Hebert et al., 2013). To establish whether acute synaptotoxicity was linked to increased behavioral output, the 7PA2 CM ICV injection model of AD was employed, and to determine whether chronic neurotoxicity was linked to increased behavioral output the bilateral IH injection model of AD (aggregated $A\beta_{1-42}$) and the bilateral IH injection model of DLB (aggregated NAC_{61-95}) were employed. The chronic IH-aggregated peptide injection models were more likely to approximate the stage of clinical dementia encountered by physicians at the point of diagnosis of dementia, and at the later stages of pathogenesis where agitation might be encountered.

The current study found that ICV injection of 7PA2 CM produced an acute memory deficit, with full recovery at 24 h after injection, as measured by incorrect lever perseverations (Fig. 1), but had no effect on RRRs, even at an extended latency (Fig. 2). This effect of 7PA2 CM on memory has been reported extensively (e.g. O'Hare et al., 2016) but not the effect on RRRs. Bilateral IH injection of aggregated $A\beta_{1-42}$ and bilateral IH injection of aggregated NAC_{61-95} produced a significant increase in incorrect lever perseverations (Fig. 3), and these findings are also in agreement with previous investigations [$A\beta_{1-42}$ (e.g. O'Hare et al., 1999) and NAC_{61-95} (e.g. O'Hare et al., 2010b)]. Incorrect lever perseverations provide a measure of the disruption of reference memory, including general aspects of executive function, reasoning, and goal-oriented manipulation of acquired information (Cleary et al., 2005; Poling et al., 2008).

RRRs are generally measured as a safeguard to ensure that decreases in response rates are not misconstrued as direct central nervous system effects. Consequently, they are usually measured to provide information on the general state of health of an experimental animal, and a fall in RRRs accompanied by a decrease in cognitive ability would indicate that any conclusion suggesting central effects might be the result of an experimental confound. IH injections of aggregated $A\beta_{1-42}$ and aggregated NAC_{61-95} resulted in memory deficits (Fig. 3), and RRRs increased following IH injections of aggregated $A\beta_{1-42}$ (Fig. 4) and aggregated NAC_{61-95} (Fig. 5). These findings indicate that the IH-aggregated peptide injection models employed here had detrimental effects on memory function and associated effects on increased behavioral output (agitation). These findings are important because, although there is one mouse model of AD in which APP23 mice have been found to show memory deficits and increased aggressiveness (Vloeberghs et al., 2006), there is very little in the literature of preclinical models of dementia that addresses the issue of memory decline and a correlation with extraneous problematic behaviors. Yet, problem behaviors, such as agitation, are highly prevalent in dementia, and specific pharmacological interventions that have not been screened against overall outcomes for the patient are frequently used in their treatment.

A recent review of investigational compounds for the treatment of agitation in dementia (Garay et al., 2016) considered the efficacy of drugs in ongoing or newly completed clinical trials. This review identified 24 clinical trials: drugs in phase III included an antipsychotic, an antidepressant, a novel compound (AVP-786), and a cannabinoid, and in phase II scylloinositol (ELND005) was likely to progress to phase III. Therefore, some headway is being made in this area. Consequently, a preclinical in-vivo screen using established procedures for modeling dementia would be of considerable value. Clinically, agitation in dementia is frequently observed

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and can range from constant vocalization, wandering, and severe sleep disturbances, but it has not been given the research emphasis applied to the basic neurophysiological etiology of memory dysfunction in dementia. However, the management of agitation, psychotic, and other non-memory-related symptoms of dementia is a major clinical concern. There is a growing recognition that neuropsychiatric symptoms, such as agitation, might increase the rate of progression of dementia. And this is particularly relevant to AD, where it has been found that neuropsychiatric symptoms, such as psychosis, agitation, and aggression, were associated with a more rapid progression to severe dementia and reduced survival times (Peters *et al.*, 2015).

A range of pharmacological agents have been employed in attempts to treat behavioral problems related to dementia; these include anxiolytic drugs, antidepressant drugs, anticonvulsant drugs, α-adrenergic and β-adrenergic drugs, lithium, hormones, and antipsychotic drugs. However, the emphasis of preclinical laboratory-based modeling of dementia has tended to concentrate on memory dysfunction, because pharmacological interventions for memory dysfunction are seen as a major research target. As a result, extension of the models employed to investigate memory to other symptoms commonly reported in the clinical literature have largely been overlooked. The findings of the current study indicate that the technique of bilateral IH injection of pre-aggregated peptides, aggregated $A\beta_{1-42}$ as a model of AD and aggregated NAC_{61-95} as a model of DLB, can be extended to provide a useful in-vivo animal model of the syndrome of agitation that is commonly associated with cognitive decline in dementia. This model could be used to investigate pharmacological interventions for agitation, including aggressive behaviors and sleep disturbances associated with the disease process. Adopting the preclinical approach to modeling agitation described in the current study would present a method for screening drugs that have ameliorative effects on emerging problem behaviors but that do not have detrimental effects on the memory functions that have already been compromised by the accumulation of neurotoxic aggregates in the brain.

Acknowledgements

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